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## GENERALIZED RETICULAR CELL SARCOMA OF LYMPH NODES ASSOCIATED WITH LYMPHATIC LEUKEMIA \*

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Leukemia, or even a leukemoid blood picture, is an unusual occurrence in the course of tumors. When the cells in the blood are morphologically identical with those of the tumor, a genetic relationship between the blood picture and the organ changes is generally assumed. If (less frequently) the leukemic cells are of entirely different structure from those of the tumor, a relationship is less firmly established. In every instance the interpretation is difficult, and the diagnosis frequently in doubt.

The case which forms the basis of this communication is one in which lesions thought to be those of an unusual tumor of the lymphatic apparatus, are associated with those of lymphatic leukemia.

### REPORT OF CASE

*Clinical History:* W. H., Shipping clerk, age 46 years. Entered Bellevue Hospital June 14, 1926, complaining of swelling on the left side of neck, duration seven weeks.

*Family History and Past Personal History:* Irrelevant.

*Present Illness:* Seven weeks ago the patient noticed a swelling on the left side of the neck which increased gradually in size. It was not painful. The patient had occasional pains in the epigastrium and suprapubic regions, of short duration, which had no relation to meals, defecation or exertion. He had lost a great deal of weight in the last two months.

*Physical Examination:* (Positive findings only.) Adult white male, appears chronically ill. Marked emaciation. Eyes: Petechial hemorrhages in palpebral conjunctivae. Neck: Masses of nodes in left cervical region, anterior and posterior chains. The individual nodes appear to be about 2 cm. in diameter. There are smaller ones in both supraclavicular regions. The nodes are firm and

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apparently discrete. Abdomen: Distended. Spleen edge 10 cm. below costal margin, firm, surface feels nodular. Liver edge at level of umbilicus, hard, smooth. Lymph nodes: Cervical, axillary, epitrochlear, inguinal, all enlarged, firm, discrete, not tender.

*Blood Examination:* Red blood count, 3,700,000; hemoglobin 60 per cent; white blood count 98,400; polymorphonuclear neutrophils 7 per cent; eosinophiles 0.2 per cent; lymphocytes 90 per cent; monocytes 2 per cent; myelocytes 0.6 per cent; plasma cells 0.2 per cent. There are many degenerated white cells in the smear. There is slight anisocytosis and pallor of the red cells.

*Clinical Diagnosis:* Lymphatic leukemia.

*Course in Hospital:* Progressive, downward. Died July 6, 1926.

#### NECROPSY

B. H. No. 11643. Summary of positive findings. There is generalized lymphadenopathy. The abdominal nodes form a retroperitoneal mass which extends from the diaphragm to the pelvis and from spleen to right kidney. The mass is composed of nodes which are 0.2 to 8 cm. in diameter, mostly discrete. Nodes in the mesentery and groin, cervical and axillary regions are also enlarged. On section all are very soft. Some are white or gray, some mottled with red, a few definitely hemorrhagic in whole or in part. Some nodes have central areas of the same consistency, but of canary yellow color. None is necrotic.

*Spleen:* 23 by 15 by 10 cm. Capsule is smooth, surface coarsely nodular. On section, there are numerous white, gray or yellow nodules measuring up to 2 cm. in diameter, of the same consistency as the lymph nodes. Many of these nodules have a hemorrhagic periphery, some have canary yellow centers. There is recent infarction.

*Liver:* Enlarged. Weight 2660 gm. It is pushed to the right and rotated by the peritoneal mass so that the left border is near the midline. There are numerous white spots on section, most less than 0.1 cm. in diameter, but some measuring up to 1.5 cm.

The nodules in the liver and spleen have the same appearance and consistency as the lymph nodes.

*Intestine:* In the ileum is a small ulcer, about 0.5 cm. in diameter, involving only the mucous membrane. It appears to be directly over a lymphoid follicle in the submucosa.

*Bone Marrow of Rib:* Hyperplastic, grayish red.

*Bones:* Normal, except for a fibroma of the periosteum on dorsal vertebra, 6.5 cm. in diameter.

## MICROSCOPIC EXAMINATION

*Lymph Nodes:* None of the lymph nodes examined is normal. Two types of histological lesions are observed:

(A) The general architecture of the node is preserved. The lymphoid tissue is markedly hyperplastic. The normal distinction between follicular and medullary areas is lost, the whole being overrun with lymphocytes, forming large areas separated by trabeculae. There are no germinal centers.

The cells are nearly all lymphocytes of the small variety. Only a few large lymphoid cells are seen. Mitoses are present, but not common.

The blood and lymph channels contain an abnormally large number of small lymphocytes. The surrounding fat tissue is infiltrated with the same cells. The endothelium and reticular tissue are normal.

(B) In the other type of lesion, there are numerous polymorphous "endothelioid" cells, sometimes closely packed, more often loosely arranged, which have the following characteristics:

The cells are several times as large as the lymphocytes. Their nuclei are large and vary in shape. Hypertrophied forms and giant cells with nuclei resembling those of megakaryocytes are present in very variable numbers.

The nuclei have definite membranes, several prominent nucleoli, and a fine chromatic network.

The cytoplasm is abundant, often with protoplasmic processes. It is moderately basophilic, particularly with the basic blue stains, less so with hematoxylin. It is finely granular, but no special leukocytic granules are present. The benzidine peroxidase reaction in these cells, as in the lymphocytes, is negative.

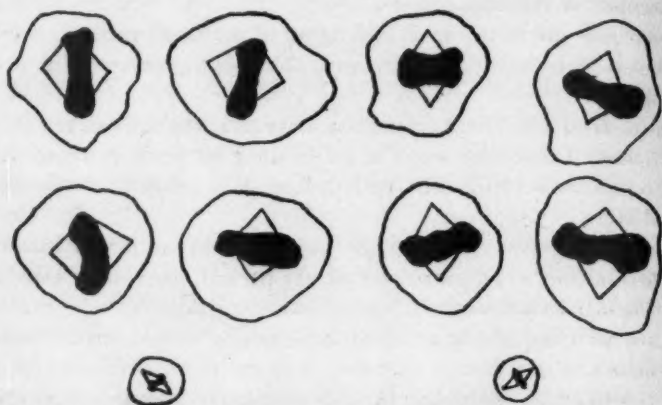
An interesting and occasional very conspicuous cytoplasmic constituent is the centrosome, which is distinctly stained, particularly by phosphotungstic acid hematoxylin (black), and by azure-eosin (red). This structure is single or double, sometimes multiple. From the centrosome are radiating lines (astral rays) which, under suitable resolution, appear granular.

The centrosome and astral rays are present not only in dividing cells, but also in many at rest. The centrosome, in cells with indented nuclei, is sometimes situated just within the nuclear indentation, or

in the cytoplasm on the same side of the nucleus. If the cell has cytoplasmic processes a centrosome may often be seen in each process if the angles are included in the plane of section. In dividing cells, a centrosome is situated in each mitotic angle.

The angle of mitosis is, on the average,  $84^\circ$  (Text-Fig. 1).

These cells are obviously of connective tissue origin, though the cells from which they arise can be determined with neither ease nor accuracy. They seem to have no genetic relationship with the



TEXT-FIG. 1. Mitoses in tumor cells and in lymphocytes, showing characteristic variations in the mitotic angles. The two small cells are lymphocytes, the others are from the tumor. Camera lucida sketch.

lymphocytes. No conversion of lymphocytes into the polymorphous cells, or vice-versa, could be found, although the two lesions are frequently found together. Neither is there any definite evidence of their origin from endothelium. Frequently they are seen just outside the lymph vessels, with intact endothelium between them and the lumen. Often, also, they are found in the situation where lymph sinuses ordinarily would be found.

I am inclined to regard these polymorphous, "endothelioid" cells as arising from the reticulum, particularly that part known as reticulo-endothelium, in the lymph nodes.

The two types of lesions are found in all the groups of nodes examined, almost always in the same nodes. Only occasionally is a section seen with only one lesion, *i.e.*, purely leukemic, or purely neoplastic. Sections through two adjacent nodes may show an

abrupt change from a leukemic to a neoplastic lesion, not an extension from one node to another.

*Liver:* The liver has the same two types of lesions that are found in the lymph nodes: leukemic and neoplastic.

The leukemic lesion consists of periportal lymphomata of different sizes, and smaller intralobular lymphocytic collections. These lesions are identical with those usually seen in lymphatic leukemia.

The other lesion consists of tumor nodules of the same cell type described in the lymph nodes, which form large areas completely destroying the liver tissue, and infiltrating the surrounding liver lobules. Sometimes these tumor cells are seen in the center of a leukemic collection, especially in the vicinity of a large tumor mass, but no transitions between the two cell types can be identified.

*Spleen:* The nodules observed in the gross specimen are composed of collections of the same type of cell described in the lymph nodes and liver as "tumor cells." These collections are mainly rather compact, completely destroying and replacing the splenic tissue. Smaller collections of the same type of cells are present in the splenic pulp.

The malpighian corpuscles are not observed, the remaining splenic tissue consisting of numerous small lymphocytes scattered without definite arrangement.

*Intestine:* The small ulcer is directly over a nodule in the submucosa which evidently was a lymphoid follicle. Only a small portion of the lymphoid tissue is present, the remainder being obliterated by tumor cells identical in appearance with those described above. The main mass of the tumor is confined to the follicle, but a few tumor cells can be seen infiltrating the mucosa. The remaining lymphoid tissue is too small in amount to determine whether or not it is the seat of leukemic change.

*Kidney:* There is hyaline degeneration of the tubular epithelium. In a few areas are poorly defined collections of small lymphocytes.

*Tumor of Periosteum:* This consists of closely packed, elongated fibroblasts and collagen fibers arranged in bundles which run in different directions. The cells have no resemblance to either the lymphocytes or to the cells of the generalized tumor. In a few places there are collections of small lymphocytes.

*Bone Marrow:* Unfortunately, examination of the marrow was confined to that of the ribs and vertebrae. In those situations, the marrow is very cellular, the predominating cell type being the small

lymphocyte. In a few areas, small clusters of myeloid cells remain. The picture is regarded as typical of the marrow in chronic lymphoid leukemia. Tumor cells were not observed.

My interpretation of this case is as follows:

The patient had chronic lymphatic leukemia for a much longer time than the history indicates. Subsequently there developed a rapidly growing, malignant tumor arising from reticular and reticulo-endothelial cells of the lymphoid tissues. The tumor developed in, encroached upon, and destroyed tissue which was previously the seat of leukemic changes.

#### EPICRISIS

*On the Leukemic Lesion:* It is known that blood pictures resembling leukemia may occur as a result of different conditions, among them tumors. It is also known that in cases with the gross and microscopic lesions of leukemia, the blood picture may give no indication of this condition. We must, therefore, regard the blood picture in leukemia (or in any other condition) as a *symptom* which may vary within wide limits and which does not in any sense constitute the disease itself. A diagnosis of leukemia based solely on the blood picture may, therefore, be questioned, but when combined with the typical features of leukemia in the various organs, it must be regarded as established.

In other words, the diagnosis of leukemia rests, in the final analysis, on tissue changes without which the disease cannot be said to exist, regardless of the blood findings. When these changes are present, leukemia may be diagnosed regardless of the apparent cause, for we are not able at present to establish the diagnosis of leukemia on an etiological basis.

Applying these statements to the present case, we may diagnose the presence of leukemia because of the typical histological changes in the organs together with a leukemic blood picture. Whether the leukemia is due to the tumor or is a disease *sui generis*, is another question.

*On the Tumor:* There can be little doubt that the lesion described in the foregoing account as a "tumor" should be so considered. The classification of the tumor, however, presents greater difficulties.

Although arising primarily in lymphoid tissues, the tumor is composed neither of lymphocytes nor their immediate precursors.

This is borne out by a study of the cells in both section and smear preparations, in neither of which do the tumor cells resemble any variety of normal blood corpuscles or their formative cells. For this reason, I think the term "lymphosarcoma" is not applicable.

The tumor is apparently derived from reticular and reticulo-endothelial cells. It has no connection with ordinary vascular endothelium, and is therefore not an "endothelioma." Certain properties normally possessed by reticulo-endothelium, however, are missing. For example, the cells do not produce reticulum fibers. In silver impregnation methods, an increase in the amount of reticulum is not demonstrated, nor is there a very intimate relation between cells and fibers. Phagocytosis is not observed, and abnormal cytoplasmic inclusions are not found in the tumor cells. This, however, does not disprove their origin from reticulo-endothelium, as it is known that tumor cells do not necessarily carry on the functions of the tissue from which they arise.

It would appear, therefore, that we are justified in regarding the lesion *in its final state* as a tumor probably arising from reticulum cells. Although it cannot be proved that the lesions in their incipency were of the same nature as those observed at necropsy, nevertheless to postulate a preëxisting lesion of another type (which has been transformed), is to read into the picture signs which are not there.

*On the Relation of Leukemia to Tumor:* The two lesions in this case have been referred to as "leukemia" and "tumor" for purposes of description. The fact that leukemia may itself be a tumor, does not enter into this discussion.

It is possible that the development of one of the lesions was dependent on the existence of the other. This question, however, involves a discussion of the etiology of both leukemia and tumors, and cannot be elaborated here. There is nothing in the foregoing account that precludes such dependence, and the fact that the tumor was encroaching on tissues formerly the site of leukemic changes, points in that direction.

The complete independence of the two lesions is conceivable, but not susceptible of direct proof. On one point, however, the histological evidence is conclusive: there is no evidence of the transformation of the cells of one lesion into those of the other. Lymphocytes and tumor cells, although intimately intermingled, are morphologic-

ally distinct. Cells in the blood stream are identical with those of the leukemic lesions, but strikingly different from the tumor cells.

This difference is also seen in a study of the "mitotic angles" of dividing cells. Ellermann<sup>1</sup> has observed that cells in mitosis have mitotic angles characteristic of the cell type. Petri<sup>2</sup> has confirmed this, and has used the mitotic angle to differentiate cell types in leukemia. Thus myeloblasts have angles of about 66° to 70°; neutrophil myelocytes about 68° to 70°; erythroblasts 21° to 22°; lymphocytes about 45°. Measurements of the mitotic angles of cells in this case showed that in lymphocytes the angle is about 45°, but in the tumor cells, 84°. There is considerable variation among the different cells of the tumor, and individual angles were found to vary from 69° to 108°, but none was found to be as low as in the lymphocytes. The average angle observed in the tumor does not correspond to any reported in blood cells.

That this case is not a "leukemic transformation" of another condition is evident from a study of the sections, which indicates that the leukemia was probably the lesion first to develop. At the time of death, the tumor was much the more actively growing lesion. The wide distribution but relative quiescence of the leukemic lesion indicates long existence and slow development.

The evidence presented by the microscopic preparations thus enables one only to diagnose the *presence* of two lesions, without giving any definite indication that they are genetically related.

I am indebted to Dr. Francis Carter Wood for the photomicrographs illustrating Figs. 3 to 8, inclusive.

#### REFERENCES

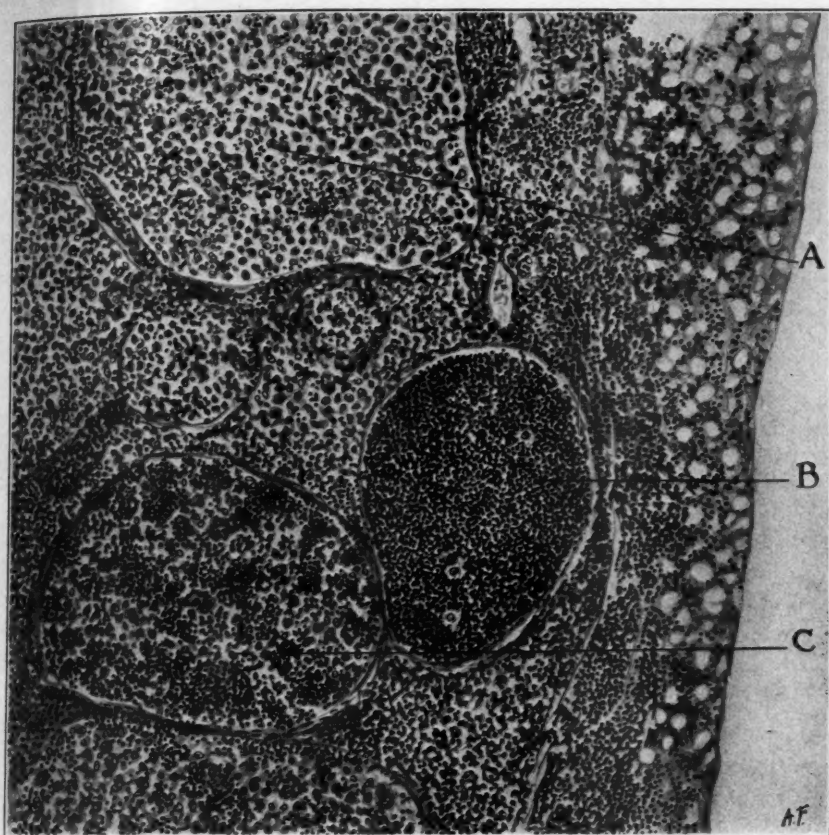
1. Ellermann, V. Messung der Mitosenwinkel als Methode zur Unterscheidung verschiedener "lymphoider" Zellformen. *Folia Haematol.*, 1923, xxviii, 207.
2. Petri, Svend. Histologische Untersuchung eines Falles von myeloischer Leukämie mit Messung der Mitosenwinkel. *Folia Haematol.*, 1926, xxxii, 103.

#### DESCRIPTION OF PLATES

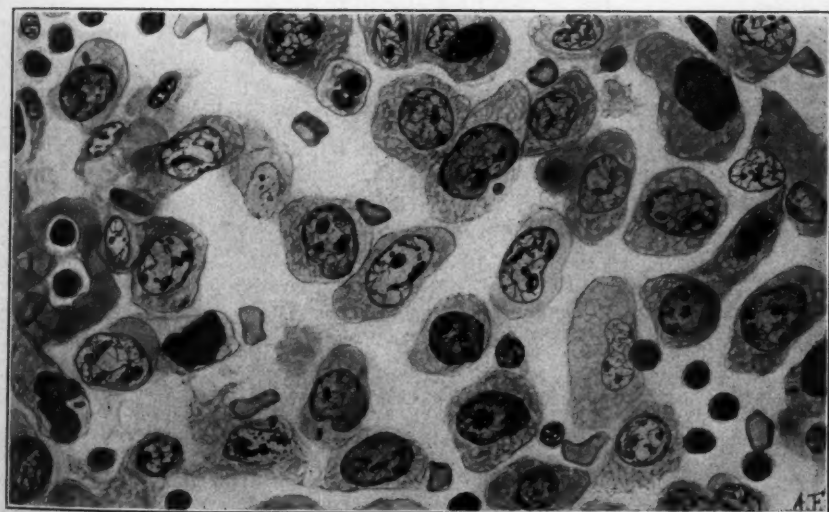
##### PLATE 70

FIG. 1. Lymph node. (A) area of almost complete replacement by tumor; (B) remains of leukemic lesion; (C) partial replacement of leukemia by tumor.

FIG. 2. Lymph node. Higher magnification of a tumor area.



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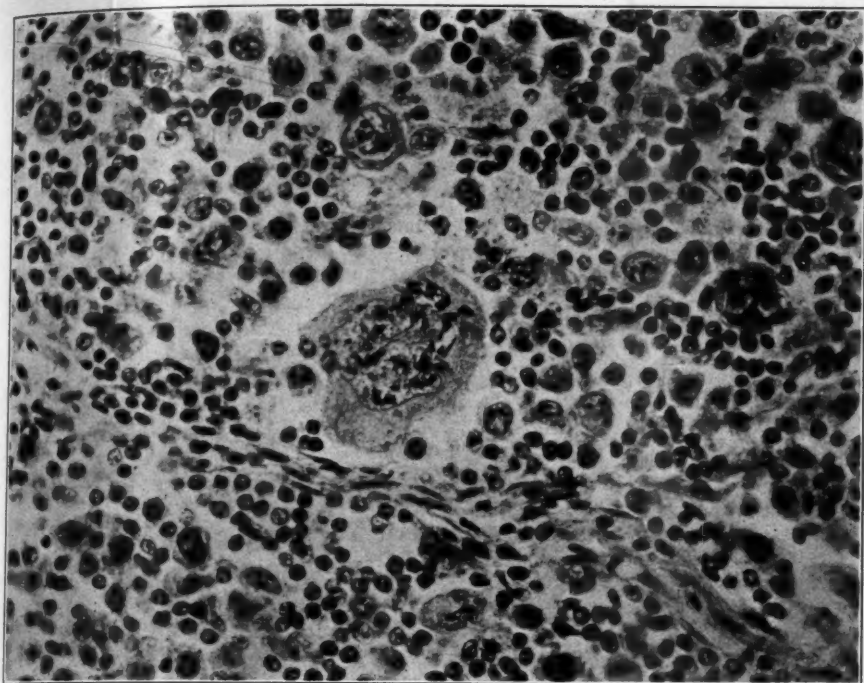
Richter

Reticular Cell Sarcoma and Lymphatic Leukemia

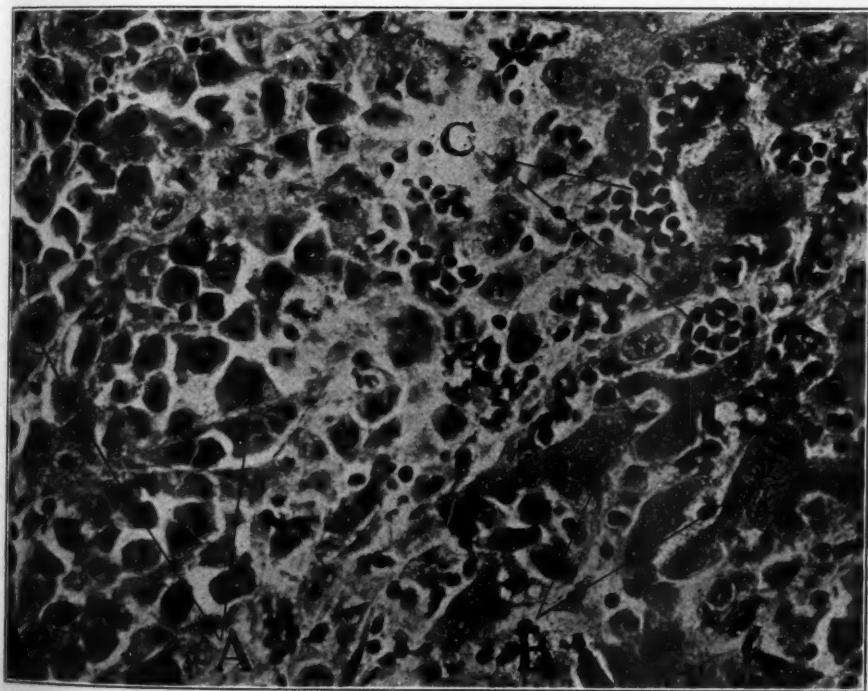
PLATE 71

FIG. 3. Lymph node. A tumor giant cell and other tumor cells intermingled with lymphocytes.  $\times 500$

FIG. 4. Liver. Edge of a tumor nodule. (A) tumor cells; (B) liver cells; (C) collections of leukemic cells (lymphocytes) in the capillaries.  $\times 500$



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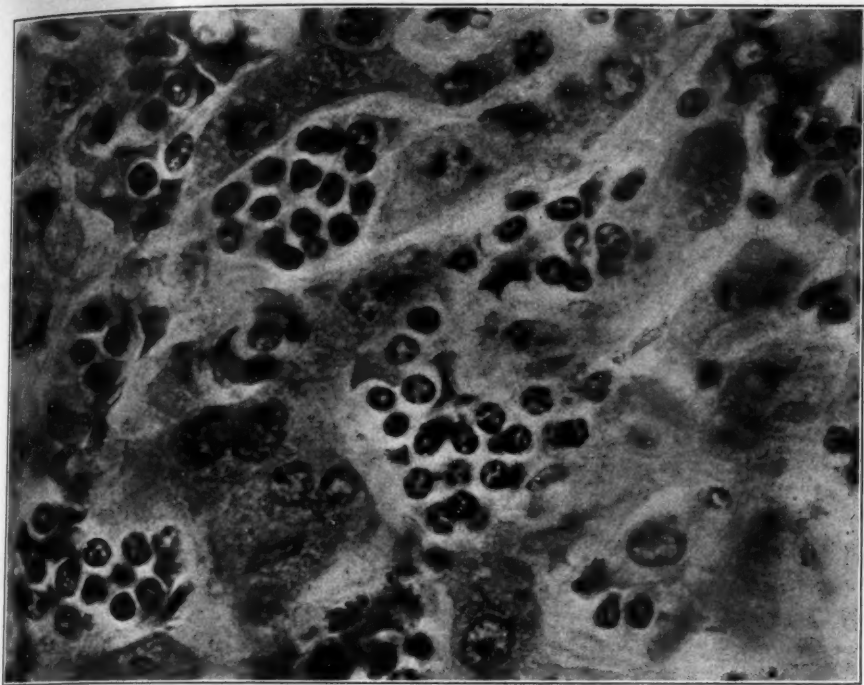
Richter

Reticular Cell Sarcoma and Lymphatic Leukemia

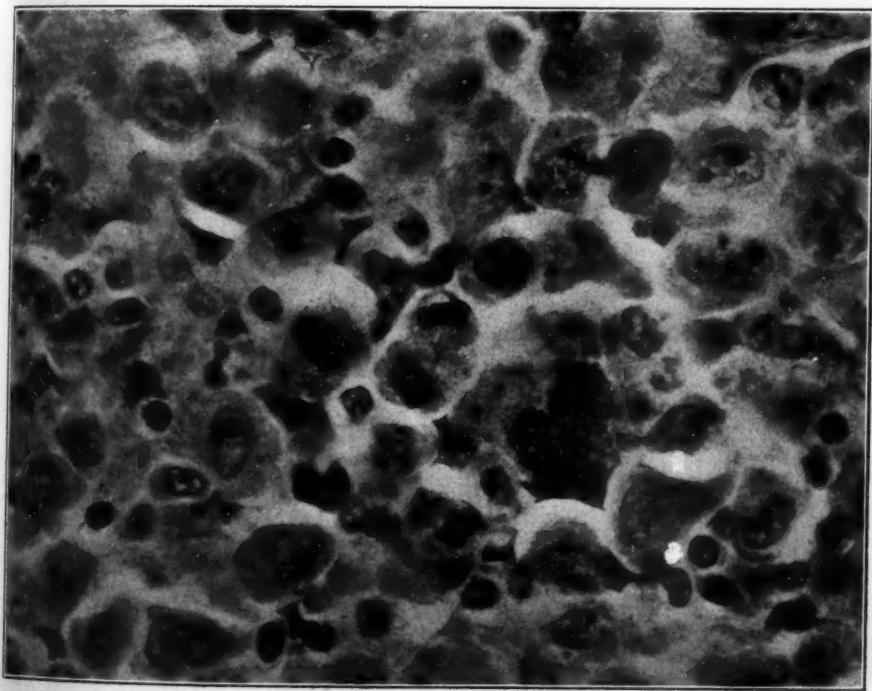
PLATE 72

FIG. 5. Liver. A higher magnification of the leukemic collections.  $\times 1000$

FIG. 6. Liver. A portion of the tumor under the same magnification as Fig. 5.  
Compare the size and structure of tumor cells and lymphocytes.  $\times 1000$



5



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Richter

Reticular Cell Sarcoma and Lymphatic Leukemia

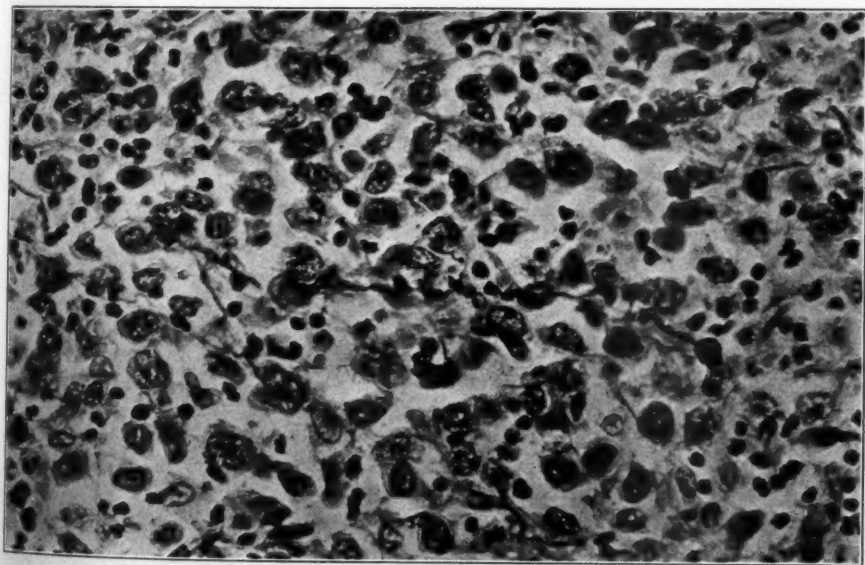
PLATE 73

FIG. 7. Intestine. Submucous nodule composed of tumor cells.  $\times 20$ .

FIG. 8. Lymph node. Stained for reticulum fibers.



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Richter

Reticular Cell Sarcoma and Lymphatic Leukemia



## STUDIES ON LIPOCHROMES \*

### IV. THE NATURE OF THE PIGMENTS IN CERTAIN ORGANS

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In previous papers the works of Willstätter and Stoll,<sup>1</sup> Van den Bergh and Snapper,<sup>2</sup> Palmer and his associates,<sup>3</sup> and others, have been referred to, and the methods, in part, by which they have established the identity of the lipochrome pigments with the carotin and xanthopyll of plants have been mentioned. I have been able to demonstrate the presence of carotin by chemical means in the liver, spleen, adrenal glands, corpus luteum, skin and fat, using a method which seems to be satisfactory for its quantitative estimation, at least for purposes of comparison.<sup>4</sup> But many tissues which are commonly said to contain lipochrome are not amenable to chemical examination because of their close association with other carotin-containing tissues, or because of the small amount present in the body. It was therefore necessary to resort to histologic methods to demonstrate the presence or absence of the pigment in such tissues. By the application of methods previously described,<sup>5</sup> and by chemical examinations, I have been able to confirm the presence of lipochrome in the tissues just mentioned but could not demonstrate it satisfactorily in the heart, ganglion cells, seminal vesicles, or in any other tissues which are commonly said to contain this substance. The pigment present in these last organs, as is well known, is a yellow to brown granular substance which is frequently tinged with fat stains, and therefore has been called lipochrome in this country, and lipofuscin in Germany. These two names are used to designate the substance in most English and American literature, but they actually represent different pigments. Borst originated the term "Lipofuscin" (Hueck <sup>6</sup>) because he thought it was derived from some sort of lipoid. Sehrt <sup>7</sup> named it "fat-binding wear-and-tear pigment" (*fett haltige Abnutzungspigment*), and Lubarsch <sup>8</sup> used this term for over twenty years. From time to time during this period the question has been taken up in Lubarsch's laboratory and varying results

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reported by Brahn and Schmidtman,<sup>9</sup> Salkowski,<sup>10</sup> and Staemmler.<sup>11</sup> Salkowski, and Brahn and Schmidtman devoted their time to chemical examinations, comparing the substance to melanin. They found that it agreed well so far as elementary analysis is concerned, with the latter pigment, both containing carbon, hydrogen, nitrogen and oxygen in about the same proportions. The pigment which they analyzed contained no iron. Staemmler, using a combined iron cyanide and silver nitrate method found that all these pigments (of heart muscle, seminal vesicle, even adrenal cortex) could be blackened and he concluded that they were melanin or forms of melanin. Block,<sup>12</sup> however, insists that melanin is formed only in ectodermal or mesodermal melanoblasts, the first found in the epidermis, retina, scattered cells in mucous membranes and the nervous system, the second (mesoderma) in the choroid of the eye, and rarely in the corium. Melanin is present also in phagocytic cells (melanophores) in the corium but is not produced by these cells. Masson<sup>13</sup> agrees essentially with this, differing only in the name of the cells which form the pigment. Melanin is found only (Masson) in cells of the nervous system, and, while these are widely scattered, they can be differentiated from others by their morphologic and staining characteristics. Wells<sup>14</sup> says that melanin is probably not found even in pathologic conditions in cells which normally do not produce this pigment.

There are therefore, as regards melanin, two schools of thought: one that melanins form a group of pigments, all closely related, some formed in cells, others as the result of degenerative processes in tissues (Lubarsch), and the other that melanin is a specific substance elaborated by specific cells which, in man, are mostly of ectodermal origin, and in lower animals are of mesodermal and ectodermal origin (Bloch, Masson, and others).

Hemofuscin is a term used by von Recklinghausen<sup>15</sup> to name the yellow pigment found associated with hemosiderin in hemochromatosis. This is a yellow-brown pigment occurring in cells (endothelial or epithelial) or in the muscle and connective tissue cells of the liver, pancreas, intestinal wall and other organs in great abundance in this disease. Von Recklinghausen described brown atrophy of the heart and liver as localized hemochromatosis because of the presence of this pigment. Lubarsch and his school never recognized the name, but from the time of Sehrt's work in 1904 until recently, they have

considered it a fat-containing "wear-and-tear" pigment and adopted Borst's term, lipofuscin, for it. Recently, as has been mentioned, Lubarsch and his co-workers have tried to prove it to be melanin. Hueck identified hemofuscin with lipofuscin in 1912 and considered it to come from a lipoid, probably fatty acid. It was not until Mallory became interested in hemochromatosis that the name hemofuscin was restored.

Mallory, Parker, and Nye<sup>16</sup> have shown that this pigment is constantly associated with hemosiderin in hemochromatosis in a large number of organs, and describe it as forming granules of light yellow pigment which do not give the iron reaction, but stain with Nile blue, fuchsin, and safranin. It is soluble in alkalis and bleaches with hydrogen peroxide. Mallory<sup>17</sup> has further found it in normal organs, in the liver, pancreas, heart and kidneys. It was found in adults above the age of 45 and was always associated with hemosiderin. He found it associated with hemoglobin and hemosiderin in the wall of a chocolate cyst of the ovary and has concluded that hemofuscin is an intermediate product between the two.

Having ruled out the possibility that this "wear-and-tear" pigment is a lipochrome, after the development of methods by which the latter pigment could be rather definitely identified, it seemed necessary to apply these and other known methods to an examination of this mysterious pigment and to correlate, if possible, the results of other workers. Therefore a review of the properties by which all the known pigments are usually differentiated was made with the following result:

1. *Melanin*: Blackened with silver nitrate; precursor positive to dopa; does not stain with fat stains; insoluble; bleaches with oxidizing agents.

2. *Derivatives of Hemoglobin*:

- (a) *Hemosiderin*: Gives the iron reaction (Prussian blue with potassium ferrocyanide and acid); not bleached.
- (b) *Hematoidin*: Crystalline character; negative reaction to stains; no iron; soluble.
- (c) *Hemofuscin* (Mallory): Stains with basic fuchsin; no iron; bleached with hydrogen peroxide.

3. *Lipochromes*: Stain with fat stains; soluble in fat solvents; are easily bleached.

4. *Lipofuscin* (Borst): Stains irregularly with fat stains; insoluble; negative to silver nitrate; bleached with difficulty.

It was obvious from the start that these properties were by no means specific. For instance, practically all pigments can be bleached, and, while most pigments may be tinged with fat stains, I have previously found that lipochrome, in particular, does not take these stains. Consequently, a revision of the methods used had to be made and a technique developed, the details of which are as follows:

#### TECHNICAL PROCEDURE

1. *Unstained Sections*: Formalin-fixed, cut on the freezing microtome.

2. *Fat Stains*: The staining of formalin-fixed frozen sections with acetone-alcohol solution of Scharlach R, and aqueous solution of Nile blue sulfate.

3. *The Dopa Reaction*: This is said by Bloch to be specific for the precursor of melanin in cells, revealing it as brown or black pigment in those cells which have a potential melanin-producing property. It does not affect, or only slightly darkens, fully formed melanin. Briefly, the technique is as follows: A 1:1000 solution of 3-4 dioxy-phenylalanine in triple distilled water which has been freshly boiled and which has a pH of 7.3 to 7.4, is made immediately before using. A buffer solution (an appropriate mixture of primary and secondary sodium phosphate) is added in sufficient quantity to keep the pH within the limits mentioned (about 2 cc. per 100 cc.). Fresh unfixed tissues cut on the freezing microtome are placed in this solution for from 16 to 24 hours at room temperature, or for 4 to 6 hours at 37° C. In using the method, sections of skin (I used mostly black guinea pig skin) are placed in the solution with the sections to be studied and examined from time to time for results. All tissues should be removed before greatly discolored and before a precipitate forms. The sections are washed in distilled water, allowed to dry in the air on a slide, cleared with xylol and mounted in balsam. Some sections were counterstained with methyl green-pyronin, as recommended by Bloch.

4. *Silver Nitrate Reaction*: Paraffin sections of formalin-fixed tissues were made at first, using Wright's rapid technique (Mallory and Wright, Ed. 8, p. 447), but considerable precipitate was always

present. Bielchowsky's method gave cleaner tissues, but also usually left some precipitate. Levaditi's method with blocks of tissue gave the most uniform results. These were made in the usual way and the first fifty or so sections discarded. Sections from the deeper layers contained little or no precipitate.

5. *Bleaching Agents*: Ferric chloride, peroxide of hydrogen, or direct sunlight were used to cause bleaching, and the reaction is recorded as positive if fading of the pigments could be caused by any process short of their actual disintegration by chemicals. Ferric chloride was used in saturated solution in 50 per cent alcohol and applied to the section on slides while under occasional observation. Hydrogen peroxide was used in 3 per cent solution into which the sections were placed for from 24 to 90 hours, sometimes changing the solution several times. Blocks of tissue were dried in sunlight before or after treating with dehydrating agents and gross observation made. (This could be applied to adrenal cortex, skin, corpus luteum, seminal vesicles and testicle.)

6. *Fat Solvents*: Acetone and chloroform were used, the chloroform after dehydration with acetone. The usual process of making paraffin sections was not relied upon to dissolve out fat and lipochrome, as frequently some of the less soluble lipoids, such as cholesterol, are left in the tissues and give false reactions with fat and basic fuchsin stains. Chloroform alone, without previous dehydration of the tissues, will not extract all the lipochrome.

7. *Alcoholic Potassium Hydroxide*: Used according to a method previously reported: 10 per cent potassium hydroxide in 70 per cent alcohol plus an equal amount of 4 per cent formaldehyde solution. The tissues are placed in this solution for from 40 minutes to 2 hours, dried on a slide, and observed directly or after mounting in glycerine. This solution will dissolve the tissue if prolonged, and one must vary the time according to the tissue under investigation. The pigment, if lipochrome, collects in small masses, or precipitates as crystals which become visible in the microscope. Melanin and hemoglobinogenous pigments are not affected.

8. *Basic Fuchsin*: This is said by Mallory to stain hemofuscin. It also stains other substances, such as lipoids and the cytoplasmic granules of tissue mast cells. It does not stain melanin. The stain was used 0.5 per cent in 50 per cent alcoholic solution upon tissues after thorough extraction with fat solvents, and most sections were

differentiated in 95 per cent alcohol until practically colorless. The pigment which stained by this method could not be decolorized by alcohol.

9. *Iron Reaction:* Mallory's method with ammonium sulphate followed by potassium ferricyanide and acetic acid was used. The sections were counterstained with basic fuchsin, and differentiated in 95 per cent alcohol.

10. *Chemical Examination for Lipochrome:* Organs were dissolved and saponified in alcoholic potassium hydroxide, dehydrated, and extracted with petroleum ether after a method previously described. In some cases the resultant pigment was identified by spectroscopic examination.

#### TISSUES EXAMINED

The source of the tissue, and the pertinent histories are as follows:

##### I. *Heart Muscle:*

1. From a six weeks' old infant, dying following an operation for pyloric stenosis; very little pigment present.
2. From a 44 year old woman with nephritis and a large heart; abundant pigment present.
3. From a man of 79, death from peritonitis following a ruptured duodenal ulcer. Weight of heart 280 gm. A fair sample of brown atrophy.
4. From a man of 81 dying of arteriosclerosis and broncho-pneumonia. Heart normal in weight; abundant pigment.

II. *Liver:* From the four cases named above, and several from guinea pigs. The latter reacted essentially the same as the human livers.

III. *Intestine:* From the men of 79 and 81 years.

IV. *Spleen:* From the four cases mentioned above and from a case of lobar pneumonia in a young man.

V. *Seminal Vesicle and Prostate:* From the old men, and a man of 21 with pneumonia.

VI. *Adrenal Glands:* Eight from various sources, all essentially normal.

VII. *Corpus Luteum:* From surgical specimens at the Peter Bent Brigham Hospital.

VIII. *Skin*: From several of the adults mentioned above, from a 3 months' old infant and from a black guinea pig. All normal tissues.

IX. *Testicle*: From two old men.

X. *Carotin Lesion*: A granulomatous lesion which followed the injection of pure carotin into the peritoneal cavity of a guinea pig.

XI. *Old Hemorrhage*: A section of ovary containing a hemorrhagic cyst, the wall of which was formed by granulation tissue and endothelial cells. A great deal of pigment was present in cells and interstitial tissues.

### RESULTS

*Heart*: In unstained sections the characteristic pigment is noted in granular yellow to brown masses at each end of the nucleus, or irregularly scattered throughout the muscle. It becomes rusty-red with Scharlach R., green with Nile blue sulfate. The color is deepened by silver nitrate and after prolonged treatment some of the dense masses appear almost black. It can be bleached after extraction with chloroform and treatment with hydrogen peroxide; is harder to bleach with ferric chloride alone. It is negative to fat solvents, alcoholic potassium hydroxide and dopa. No lipochrome could be extracted. Some of the pigment gives the iron reaction; most of it stains intensely red with basic fuchsin, but some is only tinged with the dye.

*Liver*: Unstained: yellow and brown pigment in liver cells; small amount in Kupffer cells. Scharlach R stained some pigment red, some only a rusty yellow. Blue and green pigment present with Nile blue. Much of the pigment is blackened by silver nitrate; some only moderately darkened. Most of it can be bleached. It is negative to dopa, fat solvents and alcoholic potassium hydroxide, except that some seems to have been dissolved. Much of it is stained by basic fuchsin but some is only tinged by this dye. Some gives the iron reaction. Lipochrome could be extracted (carotin 1 to 6 mg. per cent).

*Spleen, Intestine, Seminal Vesicles, Prostate, Testicle*: The reactions in these organs were essentially the same as in the heart and liver. In all the pigment was tinged red with Scharlach R, and most often green, that is to say, not stained, with Nile blue sulfate. The pigment could be darkened by silver nitrate and where dense masses of it occurred it appeared black. Most of the pigment could be

bleached out. In all, the dopa reaction, reaction to fat solvents and alcoholic potassium hydroxide were negative, except that sometimes the alkali appeared to dissolve some of the pigment. Two constant results were obtained with basic fuchsin, namely, a deep red-staining substance in droplets and granules, and a more granular material which was only tinged with the dye. In all except the testicle more or less iron-containing pigment could be demonstrated. Only the spleen yielded lipochrome upon extraction (carotin, a trace to 2.1 mg. per cent). The pigment of the interstitial tissue of the seminal vesicles appeared to be the same as in the epithelial cells. Some of this was scraped away, treated with absolute alcohol, in which it was insoluble, then taken up in dilute ammonia water in which it became soluble. This gave a positive guaiac reaction, but contamination with blood could not be excluded, though the tissue had been well washed previous to treatment. In the testicle the pigment was always present in interstitial cells, and nearly always took an intense red stain with fuchsin. A rare blue dot could be found in tissues examined for iron, but the reaction was, on the whole, negative in this organ.

*Adrenal Cortex, Corpus Luteum, Carotin Lesion:* These tissues gave the same reactions. Unstained, the pigment of the adrenal cortex and corpus luteum occurs in such fine particles that it cannot be resolved in the microscope. The tissue appears uniformly yellow, and all the pigment is obscured when fat stains are used. It is dissolved out by chloroform after dehydration with acetone, is readily bleached with ferric chloride and potassium hydroxide, and when treated with alcoholic potassium hydroxide and formalin the pigment collects in small aggregates and in visible yellow to reddish yellow crystals. It is negative to dopa. Fuchsin stains the fat in which it is dissolved, but after treatment with fat solvents neither fat nor pigment remains to be stained. Silver nitrate precipitates in the cytoplasm of the fat-laden cells, but in the carotin lesion where large masses of lipochrome occurred, a darkening of the pigment occurred with silver nitrate. The pigment, upon extraction, proved to be carotin (adrenals: carotin, 4.25 to 15.6 mg. per cent; corpus luteum: carotin, 4.1 mg. per cent; this also contained an alcohol-soluble pigment, possibly xanthophyll). No pigment could be demonstrated or extracted from the adrenals of a 3 months' old infant.

*Adrenal Medulla:* The pigment here is brown in unstained sections, takes a rusty tinge with Scharlach R, and green with Nile blue. It becomes intensely black with silver nitrate and can be bleached. It could not be seen that dopa increased the amount of pigment present, though it darkened the existing pigment somewhat. It was negative to fat solvents, alcoholic potassium hydroxide, basic fuchsin and the iron reaction. Chemical examination could not be made.

*Skin:* Yellow to brown pigment was present in many of the basal cells in the unstained section. In addition a yellow crystalline pigment could be seen in some sections in the cornified outer layer, quite sparse in most specimens and absent in some. The two pigments gave different reactions. The first, in the basal cells, was not stained by Scharlach R, but became green with Nile blue. It was intensely blackened by silver nitrate, was negative to fat solvents, alcoholic potassium hydroxide and basic fuchsin, and did not give the iron reaction. Dopa increased the amount of pigment present and slightly darkened the existing pigment. The second pigment was tinged with Scharlach R, not stained with Nile blue, was darkened slightly with silver nitrate, and unaffected by dopa. It was more easily bleached than the first pigment but both bleached finally. Most of the outer pigment was soluble in fat solvents, negative to basic fuchsin and the iron reaction, and, being already crystalline, it was not affected by alcoholic potassium hydroxide. These were obviously different pigments, the deeper being melanin, the outer, lipochrome. (Some of the outer pigment might also have been melanin, as all was not dissolved out.)

*Old Hemorrhage:* Abundant yellow and light brown pigment was present in the connective tissue and endothelial cells surrounding the cyst. Some of this was tinged red with Scharlach R, and green with Nile blue. Some of the pigment was definitely blackened with silver nitrate. It was unaffected by dopa, not soluble in fat solvents, but some seemed to have been dissolved out by alcoholic potassium hydroxide. Much of the pigment stained intensely red with fuchsin and about half gave the iron reaction. Some yellow pigment was tinged red with fuchsin and some was not stained at all. Chemical examination was not made.

## DISCUSSION

It seems somewhat difficult to bring order out of the chaotic state in which one is left after a study of the varying opinions recorded above. It is obvious that too much reliance has been placed upon what are considered to be specific histologic reactions. There are a few points, however, upon which almost universal agreement exists, and if one concedes that silver nitrate, a notoriously unreliable agent, does upon occasion darken or even blacken other pigments besides melanin, a correlation can be made which is compatible with most observed facts and with some opinions. If silver nitrate blackens melanin only, then one must conclude that this pigment is normally and usually present in the liver, spleen and hemorrhagic foci, and develops in granulomatous lesions (such as those produced by the injection of carotin), a conclusion which seems entirely unreasonable. In fact, no pigment seems to be completely unaffected by this salt, all of them showing the same brownish tinge which intercellular substances exhibit. If the pigments occur in densely packed masses the density of color is increased proportionately and the appearance of blackening becomes an optical rather than an actual effect. This is probably the case in the liver and spleen where comparatively large amounts of pigments are normally found.

All pigments except hemosiderin can be bleached, lipochrome the most readily, and practically all are lightly stained with Scharlach R. Nile blue sulfate, as pointed out by Hueck, gives a greenish appearance to pigments, not because of actual staining, but because of the mixture of yellow pigment and blue dye. These dyes, and others, seem merely to be adsorbed to the surfaces of the pigments.

The dopa reaction is not expected to differentiate pigments once fully formed. The results recorded here are therefore not conclusive, but do confirm other work in that no substance was found in heart muscle, liver and other organs, except the skin, and possibly the adrenal medulla, which was capable of forming melanin from dioxy-phenylalanine.

Fat solvents, properly used, effectively remove true lipochrome and alcoholic potassium hydroxide affects this pigment only, causing it to aggregate and crystallise from its usually dispersed particles.

The two most constant reactions have been those produced with basic fuchsin stain and the test for iron. Two reactions to fuchsin

were present; (1), an intense red produced in a substance which appeared frequently as droplets rather than granules, and (2), a red tinge in definitely granular pigment. In the hemorrhagic focus one can hardly escape the conclusion that these substances represent stages in the transformation of hemoglobin into hemosiderin, a conclusion previously reached by Mallory. The intense red pigment seems to be a semiliquid substance which by condensation becomes granular and so gradually loses its affinity for the fuchsin stain. If this is true for the hemorrhagic focus, it seems likely that it is also true in other places where a similar association of pigments is found.

This brown pigment (hemofuscin, Mallory; lipofuscin, Borst; fetthältige abnutzungspigment, Sehrt; abbaupigment, Lubarsch; alterspigment, Oberndorfer;<sup>18</sup> lipomelanin, Kutschera-Aichbergen;<sup>19</sup> sometimes lipochrome in American literature) is constantly associated with hemosiderin in the normal heart, liver, spleen, smooth muscle of the intestine, seminal vesicles and prostate; is present, with hemosiderin, in increased amount in brown atrophy of the heart and liver; increases proportionately with hemosiderin in all these organs and others in hemochromatosis (Mallory); and is always associated with hemosiderin in local hemochromatosis (pseudomelanosis, ochronosis) of the large intestine (Lubarsch). Hemosiderin was present in very small amount in the testicle, and not at all in the heart of the 6 weeks' old infant, though pigment which stained with fuchsin was present in both these organs.

A word of caution is needed here. One assumes, without justification perhaps, that most material of a granular nature which is shown to contain iron is hemosiderin (that is, iron derived from hemoglobin). Where this substance is found associated with hemorrhage, the conclusion that it comes from hemoglobin seems obvious. But Sprunt, Colwell and Hagan,<sup>20</sup> have apparently shown that iron may be derived from other proteins during autolysis, and that an iron-containing pigment is not necessarily a product of hemoglobin decomposition. Hueck has said that hemosiderin is, in fact, an inorganic iron compound by the time it is recognizable in tissues, and so has no property by which its origin can be traced. The only point one can make here is that, whatever the source, it seems to be the same for both hemofuscin and its iron-containing satellite.

True lipochrome (carotin) can be demonstrated histologically in the adrenal cortex, the corpus luteum, in atheromatous plaques of

the aorta and the skin. It cannot be differentiated from other pigments in the liver and spleen, although it was shown to be present in these organs by chemical examination. Also, because of the nature of the tissue, it is not demonstrable histologically in fat, but can be extracted from adipose tissue. It is to be noted that lipochrome was not present in any of the tissues of the infants studied.

This pigment should offer no morphological difficulty. Only in the outer layers of the skin can it be seen as a granular pigment. In all other places it occurs in solution in lipoids, giving the tissue a yellow coloration, but is not visible as a particulate substance by the microscope. The granules in the skin are probably formed by condensation and precipitation in the outer layers as the epidermal cells are pushed outward and become keratinized. It is difficult to find the pigment in this tissue probably because it is actually scant in normal skin, and because a large part of it becomes oxidized and is then invisible.

Melanin could be demonstrated beyond a reasonable doubt only in the skin, although a definite effort to locate this pigment in all tissues where it might occur was not made. The pigment of the adrenal medulla gave all the reactions of melanin, and none of those which have been found to distinguish other pigments. If this work and that of Bloch and Masson are conclusive, they show that melanin is a constituent of certain special cells only, and cannot be regarded as the product of degenerative processes in tissues.

#### CONCLUSIONS

A revision of the characteristics of these several pigments must be made. So, we have:

1. *Melanin*: A brown pigment occurring in certain cells of ectodermal or nervous tissue origin (ectodermal melanoblasts) and, in man, in what are essentially cell rests, in cells of mesodermal origin (mesodermal melanoblasts) which form Mongolian spots and certain blue nevi. In addition it may occur in phagocytic cells (melanophores, chromatophores) in the corium. There is no definite evidence that true melanin occurs elsewhere. The pigment is intensely blackened by silver nitrate and is revealed, in cells which are capable of forming it, by the dopa reaction. It is insoluble in acid and alkaline solutions, and in fat solvents. In common with all other pig-

ments except hemosiderin it is bleached by oxidizing agents. It occurs pathologically in melanotic tumors.

2. *Hemosiderin*: An iron-containing pigment, insoluble, may be darkened with silver nitrate, does not bleach, stains irregularly with fat stain, is lightly stained with basic fuchsin. It occurs in phagocytic cells and intercellular tissues (after degeneration of the cells which originally contained it), normally in the spleen and liver (epithelial and Kupffer cells), and in advancing age in the muscle of the heart, intestine, seminal vesicles, prostate and blood vessels. It is increased in the heart and liver in brown atrophy of these organs; and in these and many other organs in hemochromatosis; is present in the mucosa, submucosa and muscle of the intestine in pseudo-melanosis, or local hemochromatosis. It is always present in the vicinity of old hemorrhages.

3. *Hematoidin*: Yellow crystalline or semiliquid (dissolved in tissue juices), soluble, is darkened with silver nitrate, bleaches readily, contains no iron and does not stain with fat or basic dyes.

4. *Hemofuscin*: Yellow or brown semiliquid or crystalline; insoluble in fat solvents, partly soluble in alkali; does not give the iron reaction, but darkens with silver nitrate; stains lightly with fat stains (by adsorption); in early stage of formation it stains intensely with basic fuchsin, but as it becomes crystalline, it stains only lightly with this stain, and bleaches less readily than hematoidin, melanin and lipochrome. *Hemofuscin is present in all places and under the same conditions as hemosiderin*, in usually greater amount. It, with hemosiderin, constitutes the "wear-and-tear" pigment of the body, and is probably a product of the metabolism of hemoglobin, both from blood and muscles.

5. *Lipochrome (Carotin, Xanthophyll)*: A diffuse, finely dispersed yellow pigment, associated with fat. It does not stain with fat stains, is soluble in fat solvents, bleaches readily and darkens with silver nitrate (when crystalline). It is aggregated, or becomes crystalline, when treated with alcoholic potassium hydroxide. It occurs naturally as crystals only in the outer layers of the skin. It is present normally in the adrenal cortex, corpus luteum, liver, spleen, fat and skin; increases in these tissues with increased ingestion of food, or when, for some reason, lipemia is present. It occurs in pathologic processes in atheromatous plaques of arteries, and in xanthomas. It is not present in the tissues of infants.

There are therefore two pigments which accumulate in the body tissues in advancing age, and seem to be increased in cachectic conditions. Only one of these, however, (hemofuscin, which apparently slowly becomes hemosiderin), can be regarded as a "wear-and-tear" pigment, the result of slow or rapid disintegration of a tissue protein. (The fact that this pigment is present in the heart of infants is dismissed by Rössle<sup>21</sup> with the statement that "Man is old before he is born.") When lipochrome is present in increased amount it is due to increased ingestion, or to the absorption of the fat containing it. In the latter condition, no more lipochrome is present than formerly, but the proportion to the amount of remaining solvent is greater.

#### SUMMARY

A review of the characters which are said to differentiate the various pigments of the body was undertaken in conjunction with the chemical examination of certain organs for lipochrome (carotin and xanthophyll). Tissue from the normal heart, liver, spleen, skin, intestine, seminal vesicles, prostate and testicle, as well as a lesion produced by carotin injection in a guinea pig and an old hemorrhagic focus, were treated in identical ways histologically, and where feasible, chemically. Two groups of pigments were found to accumulate in the body with age, namely, lipochrome and hemofuscin (with hemosiderin). The former of these is exogenous in origin; the latter constitutes the real "wear-and-tear" pigment, and possibly is derived from muscle hemoglobin.

From the results of this inquiry the pigments present in the various tissues studied seem to be:

1. *Skin*: Melanin and lipochrome; also, in hemochromatosis; hemofuscin and hemosiderin.
2. *Fat*: Lipochrome.
3. *Heart, Intestinal Muscle, Seminal Vesicles, Testicles, Prostate*: Hemofuscin and hemosiderin. Increased in old age, brown atrophy and hemochromatosis.
4. *Liver and Spleen*: Hemofuscin and hemosiderin besides bile pigment. Increased in old age, brown atrophy and hemochromatosis.
5. *Adrenal Cortex*: Lipochrome.
6. *Adrenal Medulla*: Probably melanin.
7. *Corpus Luteum*: Lipochrome.

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SOME POINTS ON THE MECHANISM OF FILTRATION BY  
THE SPLEEN \*

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Among the many and varied functions attributed to the spleen, that which stands out most prominently is its ability to filter out from the flowing blood stream foreign particulate matter such as broken down red and white blood cells, platelets, carbon and iron pigment, and other materials. This function was recognized by Ponfick in 1869,<sup>1,2</sup> who found that it held back not only degenerating red blood cells but also other foreign substances. He spoke of this function as being "spodogeneous" in nature. Frey,<sup>3</sup> and Rautman,<sup>4</sup> confirmed these observations that the spleen possesses the function of filtration by finding fewer red cells in the splenic vein than in the artery. This removal of red blood cells by the spleen they found was related to the change in the osmotic resistance of the red cells. When this resistance was increased by phenylhydrazin the number of cells in the vein equalled that in the artery, while on the other hand, if it was lowered by ether the difference in number became very much greater. Frey also showed that foreign red blood cells introduced into the splenic artery were held back in the pulp. Opie<sup>5</sup> got similar results by the transfusion of blood from one animal to another. Bedson<sup>6</sup> has shown also that the spleen regulates the number of platelets in the circulation, taking up and destroying those which are of no further use. Voorhoeve<sup>7</sup> regarded the spleen as a filter for all abnormal materials present in the blood.

Considerable experimental work has also been done to prove this filtering function of the spleen on the circulation. Wyssokowitsch,<sup>8</sup> the first to make intravenous injections of bacteria and to note their distribution, found that many of them were caught in the spleen. Bouffard<sup>9</sup> and Goldman,<sup>10</sup> with intravenous injections of dyes, found that in part they were deposited in the spleen. Duhamel,<sup>11</sup> using colloidal solutions of various metals and Foot,<sup>12</sup> Tait and

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McCartney,<sup>13</sup> and Nagao,<sup>14</sup> using India ink for intravenous injections, found considerable deposits in the spleen. Drinker and Shaw,<sup>15</sup> using a suspension of manganese dioxide, got similar results. These observations and experiments tend to show that the spleen is somewhat of the nature of a filter for the blood stream, being able to remove from it particulate matter of a foreign nature.

The ordinary conception of a filter is a device for mechanically straining out particulate matter from a fluid menstrum. While this may be true in a general way, the mechanism by which filtration takes place is often very complex. There are a number of variable factors which will determine whether or not a given substance will be strained out. The size of the particle in relation to the size of the pores of the filter is an important one. If the particles are larger than the pores, the process of course is quite simple, being merely a mechanical separation of the particles from the menstrum. On the other hand, in the case of minute particles of a colloidal solution and even of bacterial suspensions, when such filters as the Berkefeld or Chamberlain are used, mechanical separation no longer plays the important part. These filters were long thought to separate the bacteria from their suspending fluids, by virtue of the small diameter of the pores. Bechhold,<sup>16</sup> for instance, in 1908, calculated the mean diameter of the larger pores of a new Chamberlain "F" filter, to vary between 0.23 and 0.41 microns. Mudd,<sup>17</sup> using Bechhold's formula, found the average diameter of Berkefeld filters of the "V" type to be 0.38 microns, the "N" type to be 0.45 microns, and the "W" type to be 0.43 microns. As most bacteria are larger than these diameters the process of filtration appeared to be mechanical in nature. Bigelow and Bartell,<sup>18</sup> however, in 1909 pointed out an error of one decimal point in Bechhold's formula. The corrected formula showed the pores to be ten times larger. Using the figures given above and allowing for the error in the Bechhold formula the mean diameter of the pores of the Chamberlain "F" filter should have been 2.3 to 4.1 microns and in the case of the Berkefeld filters 3.8 microns for the "V" type, 4.5 microns for the "N" type and 4.3 microns for the "W" type. These diameters are greater than those of most bacteria and should offer no hindrance to their passage.

The work of Mudd<sup>17</sup> on the passage of cultures of *Vibrio percolans* and that of Wolbach, Binger, and Todd,<sup>19</sup> on the passage of certain spirochetes through Berkefeld filters shows that there is no reason

based on mechanical obstruction why bacteria should not pass through. Bulloch and Craw<sup>20</sup> found many Berkefeld filters even at atmospheric pressure permeable to bacteria. However, this is not generally true for with good filters most bacteria are held back, there being only a few strains which can consistently be passed through.

Berkefeld and Chamberlain filters, having such large diameters to their pores, obviously do not mechanically separate bacteria from the filtrate. Neither does motility of the bacteria play a part, for Mudd<sup>17</sup> was unable to pass the much more motile organism, *Vibrio comma*, through a filter which previously passed *Vibrio percolans*. As for the mechanism of filtration, Mudd,<sup>21 22</sup> offered the suggestion that it was dependent upon electrical attraction between the bacteria or particulate matter and the wall of the filter. He described the surface of the pores of a Berkefeld filter as the site of an "electrical potential difference," a helmholtz double layer. The wall of the filter, he believed, carried a negative charge and the adjacent liquid a positive charge. He predicted and found that positively charged particles in suspension were adsorbed and retained by the filter while negatively charged particles passed through. *Vibrio percolans* were found to be negatively charged and as the wall of the filter carries a negative charge, the bacteria were repelled and passed through. This conception was further substantiated by Kramer<sup>23</sup> who made a filter that was oppositely charged. He made it with commercial plaster of Paris. The positive charge on the walls of its pores depended upon the presence of calcium carbonate in the mixture and was further enhanced by adding up to 25 per cent of magnesium oxide, calcined at 1300° C. He now possessed a filter of opposite charge to that of the series of siliceous filters such as the Berkefeld and Chamberlain. He observed that with this he could remove from the menstrum all colloids and suspensions which passed through Berkefeld filters and *vice versa* all substances which passed through the plaster of Paris filter were readily held back by the siliceous filters. He then constructed a combined filter, using a Berkefeld filter for a core and a plaster of Paris filter for a cortex. Colloids both positively and negatively charged were removed by this combination filter.

## STRUCTURE OF THE SPLEEN

The internal structure of the spleen is such as to suggest that it is primarily a filter. It is essentially a spongy mass of reticulo-endothelial cells belonging to the system as described by Aschoff.<sup>24</sup> It is surrounded by a capsule and supported by a trabecular framework of smooth muscle and elastic fibers with a connective tissue stroma. This framework serves to control the flow of blood through the organ. The blood is widely distributed by an arterial system which divides and terminates in end capillaries. The latter have been shown by the author<sup>25</sup> to open out and discharge their contents into a vast cavernous network of pulp spaces formed by the above mentioned reticulo-endothelial cells as they stretch across the intervening spaces of the trabecular framework. The circulation is continued into the venous channels through slit-like stomata in the walls of the veins. The circulation is therefore open. The end capillaries are unique in that toward the ends they are ensheathed in a condensed mass of pulp cells called ellipsoids. These will be shown later to play an important part in the mechanism of filtration.

The spleen differs from ordinary filters, such as the Berkefeld, in the fact that the size of its pores varies with relaxation and contraction of its muscular system and the flow of fluids through its pores is more or less intermittent, being controlled by this same muscular system. It is similar however in the fact that the suspended material is brought directly in contact with the walls of the pores, namely, the pulp ellipsoid cells. This is brought about first by the devious courses taken by the blood through the maze of pulp spaces and secondly by the stagnation of the blood during periods of relaxation of the muscular system. The chance of contact of pulp cell and particle has been dealt with from a theoretical standpoint by McKendrick<sup>26</sup> and experimentally by Fenn.<sup>27</sup> The latter has shown that if a suspension of cells which have a diameter "C" and velocity (under gravity)  $V_c$  and of particles of a diameter "P" is allowed to settle in a test tube the chances of collision "R" between them will be proportional to the velocity of the particle to the cell and to the square of the sum of their diameters or  $R = (V_p - V_c) (C + P)^2$ .

In the case of the spleen, however, the particles alone are in suspension, the filtering cells (pulp and ellipsoid cells) being fixed and having no velocity.  $V_c$  therefore is eliminated. The above formula

is also based on the supposition that the cells and particles are spherical in outline. This represents the minimum surface area for a given mass. If the pulp cells were spherical in outline the chances of collision "R" between foreign particles in the flowing blood through the spleen and the pulp cells would be proportional to the velocity of the blood to the square of the sum of the diameters of the particles and of the pulp cells. The surface area of the pulp cells, however, is tremendously increased by the fact that their cytoplasm is stretched out into long filamentous processes. The chances of contact of particles in the flowing blood with pulp cells is thereby considerably increased over that represented by the formula.

Having in mind the mechanism of filtration in Berkefeld and other filters a number of experiments were carried out to answer the following questions: (1) Does filtration occur in isolated as well as in normal spleens? (2) Can the filtrate be dislodged? (3) Is filtration in the spleen mechanical in nature? (4) Is filtration in the spleen dependent upon living cells? (5) Is filtration in the spleen electro-physical in nature?

#### EXPERIMENTS

Sheep, dog, and cat spleens were used. The filtrating fluid was either perfused through the freshly isolated spleen or injected intravenously and the spleen removed later. As substances for filtration India ink, carmine, acid and basic dyes, and colloid solutions of copper, platinum, and silver were used.

With freshly isolated spleens the anastomotic circulation was tied off and canulae inserted into artery and veins. It was found advisable to suspend the spleen in a basin of warm water while the injections were being carried on. Following, and sometimes also before, they were perfused with either distilled water or normal saline solution. The injections into the artery were made with a pressure varying between 60 mm. and 150 mm. Hg and a back pressure in the vein of 10" of water. These pressures were found sufficient to distend the spleen to capacity without in any way damaging its delicate network of cells. To provide for a proper flow it was found absolutely essential to eliminate all bubbles of air from the filtering and perfusing fluids, otherwise they acted as emboli and blocked the

circulation. If sufficient pressure was applied to dislodge them they caused gross lacerations of the delicate pulp tissue.

The first series of experiments were done to determine whether filtration occurred in freshly isolated spleens as well as *in vivo*. Fresh sheep, cat, and dog spleens were isolated, anastomotic circulation tied off, and the canulae inserted into the vein and artery, and the whole organ suspended in warm water. Ten cc. of a 1 per cent solution of Higgins' India ink was injected into the artery. It was then perfused with warm normal saline at a pressure of 100 to 150 mm. Hg until the return fluid from the vein was clear. Twenty per cent formalin was perfused in the same manner until the spleen was thoroughly bathed with the fixative. While in the distended state ligatures were applied to the artery and vein. Gross and microscopic sections showed that filtration had occurred, for adherent to the filamentous processes of the pulp cells were large deposits of India ink. Perfusions of distilled water, both before and after the India ink injections, failed to alter the filtering process. Intravenous injections of similar solutions showed again that the spleen possessed the ability to separate India ink particles from the blood for considerable deposits were found on the ellipsoid and pulp cells. These experiments further demonstrated that even with prolonged perfusion the ink particles could not be dislodged by normal saline or distilled water. It was found advisable to make the perfusions with distilled water as the saline solution caused the fine particles of ink to conglutinate and settle out in small masses.

The mechanical factor in the filtration would seem to be eliminated by the fact that the pulp spaces are much larger than such colloidal particles as India ink. The pulp spaces have been shown by the author<sup>24</sup> to vary between 0.005 to 0.02 mm. in diameter. However, to substantiate this, freshly isolated sheep, cat, and dog spleens were perfused with warm normal saline at a pressure head of 100 mm. Hg. in the artery and a back pressure of 10" of water in the vein. This caused the spleen slowly to expand to its capacity, and as has been shown before to dilate all the pulp spaces to their full diameter without rupturing the pulp cells. The vessels and pulp spaces being dilated to capacity a 1 per cent solution of India ink was slowly introduced into the perfusing fluid, and perfusion continued until return flow. After these injections the spleens were perfused with 20 per cent formalin until thoroughly impregnated

with the fixative. Gross sections showed that filtration was very effective, the pulp tissues being quite dark gray in color. Microscopically, the vessels and pulp spaces were found to be dilated to capacity and free from any particles of ink. The filamentous processes of the pulp cells however, were covered with fine deposits of ink particles. It is quite apparent therefore that mechanical separation by virtue of the size of the pores in relation to the size of the particles of ink is eliminated.

By the foregoing experiments it has been shown that the spleen is able to filter out India ink particles from the blood plasma, normal saline solutions and distilled water. The distilled water must have interfered with the vitality of the pulp cells. This, however, did not hinder the process of filtration. To prove more definitely that filtration by the spleen is not dependent upon the vitality of its cells, two freshly isolated sheep spleens were perfused, first with normal saline, then with 100 cc. of a 1 per cent solution of sodium cyanide. Perfusion with normal saline was continued for a few minutes, then 40 cc. of 1 per cent India ink solution was introduced. Filtration appeared to occur just as readily as in the previous experiment. This was verified by microscopic examination. Large deposits of the ink particles were found adherent to the filamentous processes of the pulp cells. Filtration by the spleen, therefore, is not dependent upon the vitality of its cells.

Having in mind the factors which determine filtration in Berkefeld and plaster of Paris filters, it was thought advisable to test these on the spleen. While up to the present only a few experiments have been made, they seem to indicate that the mechanism of filtration is selective in character and dependent upon the electrical charge of the suspended particles. Colloidal solutions (Bredig) of platinum, silver, and copper were used. Colloidal particles of platinum and silver are negatively charged, while those of copper are positively charged. Perfusion experiments with isolated spleens were not found to be very satisfactory. This was particularly true when normal saline was used. The colloids were precipitated almost immediately in the presence of the salt and were deposited upon the walls of the larger vessels.

The results obtained by first perfusing with distilled water were somewhat better. A freshly isolated spleen was perfused first with distilled water then injected through its artery with 175 cc. of a

colloidal silver solution (negative) and perfusion continued for ten minutes. The second spleen after first perfusing with distilled water was injected with 110 cc. of colloidal platinum solution (negative) and followed by further perfusion with distilled water, as in the first case. The third spleen was perfused in the same manner as the first and second, but 325 cc. of a colloidal copper solution (positive) was injected into the artery and perfusion continued. After fixation, sectioning and staining it was found that the first and second spleens respectively had filtered out the platinum and silver. Particles were found adherent to the pulp cells as seen in the illustrations. In the case of the third spleen, no evidence of copper deposits could be found, either directly or after Perle's reaction. This latter reaction should show the copper as a brown precipitate.

In order to overcome the tendency of these colloids to precipitate on to the walls of the large vessels and to take advantage of the protective colloids of the blood plasma, intravenous injections were tried.

A dog weighing 3.5 kg. under an anesthetic during a period of nineteen minutes was injected intravenously with 325 cc. of a colloidal copper solution (positive). The dog died immediately after the injection. Blocks were taken from various areas of the spleen and closely examined for traces of copper deposits, but none was found.

A cat weighing approximately 2 kg. was then anesthetized and during a period of seven minutes was injected intravenously with 60 cc. of a colloidal platinum solution (negative). After nineteen minutes from the commencement of the injection the animal was killed and the spleen fixed and sectioned as in the previous case. Microscopically, fine deposits of the platinum particles could be seen upon the cells of practically all the ellipsoids.

#### COMMENT

While it has long been known that the spleen filters out foreign materials from the blood stream, the mechanism of this process has not been clearly demonstrated. In the case of Berkefeld and similar filters it was formerly thought to be a mechanical separation by virtue of the small diameter of their pores. Bechhold's<sup>18</sup> formula for estimating these diameters would seem to substantiate this view but more recent experimental work and the calculations of Bigelow

and Bartell<sup>18</sup> showed that the process could not be one of a mechanical nature. Mudd has suggested that filtration by Berkefeld filters is dependent upon adsorption. He believed the walls of the pores to be the site of an electrical charge and to adsorb all particulate matter carrying an opposite charge. The work of Kramer would further substantiate this view.

I have shown that in the case of the spleen, either isolated or *in vivo*, the possibility of mechanical separation of foreign particles from the flowing blood is almost impossible because of the relatively large size of the pulp spaces compared with that of the particles.

The process is not a vital one as filtration occurs just as readily in the isolated spleen whose cells have been rendered functionless by cyanide as in the living spleen.

The mechanism of filtration by the spleen is apparently the same as that of such filters as the Berkefeld, Chamberlain, and plaster of Paris filters. It is one of adsorption of the particle to the wall of the pores or in the case of the spleen it is an adsorption of the foreign particles to the pulp cells. This is a non-vital process and is dependent upon the relation of the electrical charge of the suspended particle to the electrical charge of the surface of the pulp cells. When the charges are relatively the same the particles and cells repel each other and the particles pass through the spleen. When the charges are opposite, the particles and cells are attracted and the adsorption occurs. Filtration is selective in character and dependent upon the electrical charge of the suspended particles. Of course the further process of phagocytosis is another problem and brings in new factors not dealt with in this study.

The work of Duhamel,<sup>11</sup> on the intravenous injections of various colloidal solutions and the analysis of the organs for their recovery, would seem to substantiate this theory of selective filtration. He gave intravenous injections of colloidal silver, platinum, and copper, as well as other colloidal solutions. The exact amount of each metal by weight was determined before injection, then a chemical analysis of the various organs was made to determine the distribution. The silver and platinum (negatively charged) deposits were found chiefly in the liver with traces in the spleen. The copper (positively charged) deposits were found chiefly in the blood with slight amounts in the liver and none in the spleen.

Jancso<sup>28</sup> has recently made use of this selective affinity of the

reticulo-endothelial system for negatively charged particles to carry certain positively charged colloids to these cells. He used an inert negatively charged colloid like Chinese ink as the vehicle for the drug and by intravenous injections he was able to produce profound changes in this system.

#### SUMMARY

1. The spleen is essentially a blood filter, filtration occurring equally well in the isolated spleen as in life.
2. The process of filtration is not dependent upon the relative size of the spaces to that of the particles, nor is it dependent upon the vitality of the pulp cells.
3. The process is apparently electro-physical in nature and depends upon the electrical charge of the particle in relation to that of the pulp cell.
4. The adsorbed particles cannot be readily dislodged from the surface of the pulp cells.
5. The spleen filters out negatively charged colloidal particles.

I am indebted to Prof. E. F. Burton, both for the colloid solutions used and for much helpful advice, and to Prof. Oskar Klotz for his help in the supervision of the experiments.

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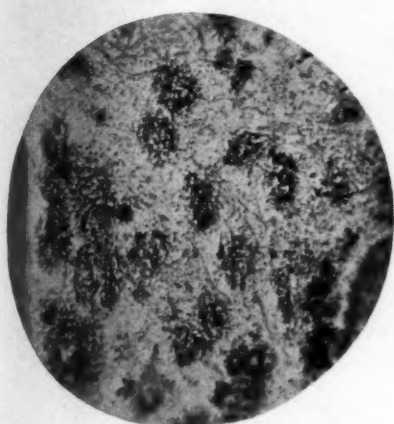
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## DESCRIPTION OF PLATES

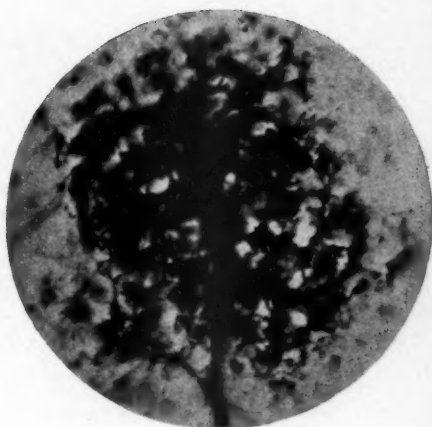
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### PLATE 74

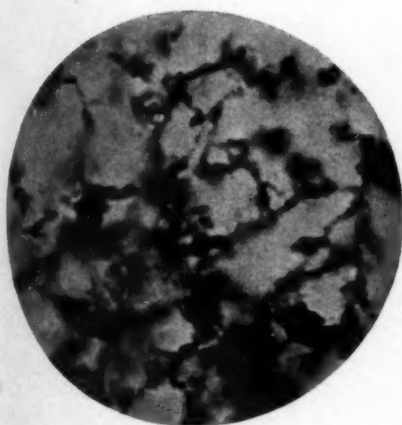
- FIG. 1. Isolated sheep spleen perfused with small quantity of India ink solution. India ink particles adherent to pulp cells about the end capillaries.
- FIG. 2. Higher power showing the India ink filling the end capillary and adherent to the pulp cells about it.
- FIG. 3. High power showing the India ink adherent to the filamentous processes of the pulp cells.
- FIG. 4. Dog spleen, distended to capacity, then perfused with India ink solution. The ink is shown adherent to the walls of the end capillaries and to some of the neighboring pulp cells.



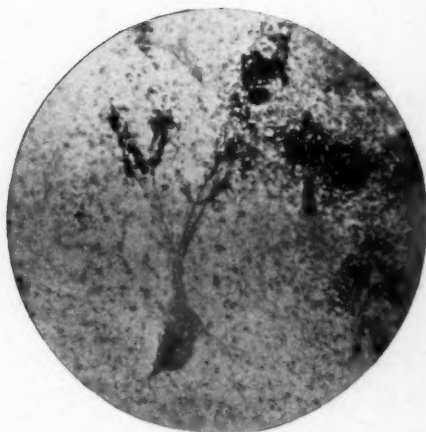
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Robinson

Mechanism of Filtration by Spleen

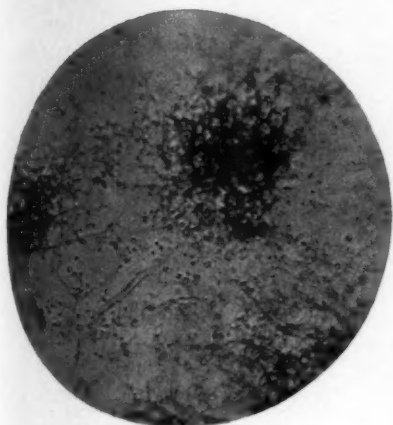
PLATE 75

FIG. 5. Sheep spleen, perfused first with potassium cyanide solution, then India Ink injection. The ink particles have adsorbed to the pulp cells about the end capillaries.

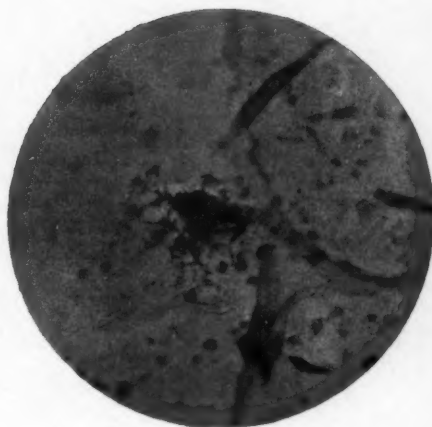
FIG. 6. Colloidal silver (negative charge), solution was perfused through a sheep spleen. The silver particles have adsorbed to the pulp cells.

FIG. 7. Same as Fig. 6.

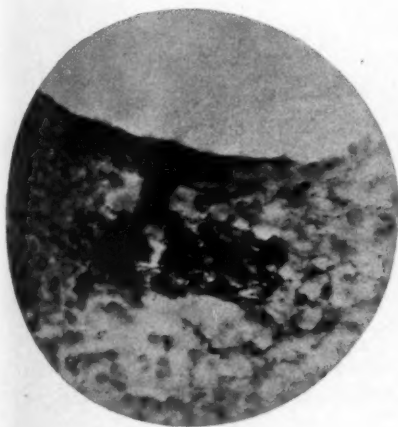
FIG. 8. Sheep spleen perfused with colloidal platinum (negative charge), solution. Platinum particles have adsorbed to the pulp cells.



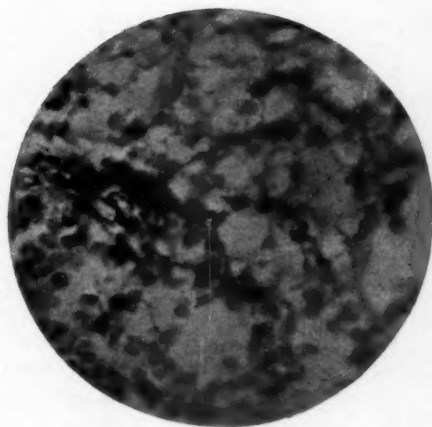
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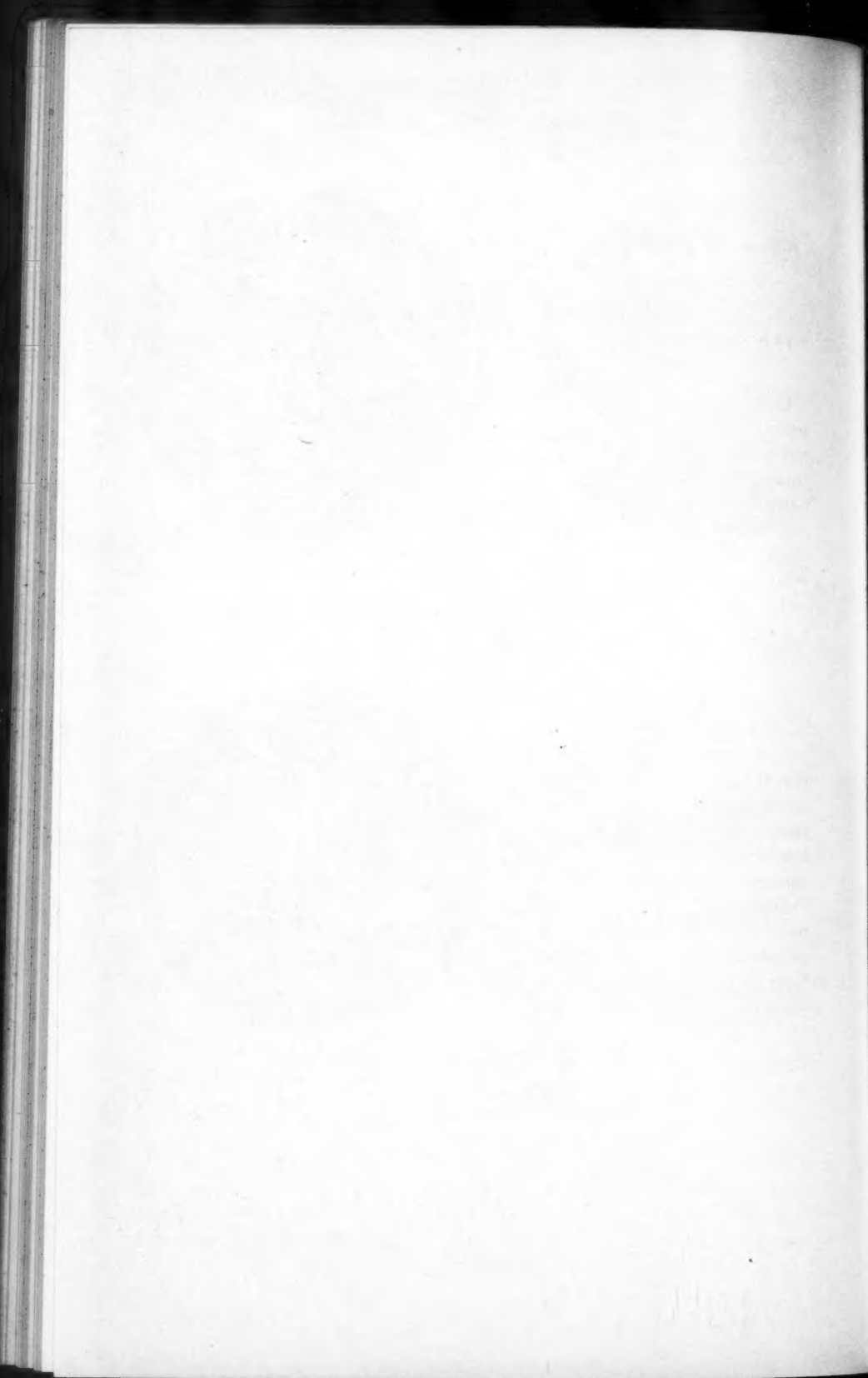
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## HUMAN MERCURIC CHLORIDE POISONING BY INTRAVENOUS INJECTION \*

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Cases of mercury poisoning have frequently been observed and reported in which the poisoning followed ingestion, inhalation, absorption from inunction, douches, wound irrigations and subcutaneous or intramuscular injection. Cases terminating fatally following administration by any route other than by the mouth are rare and we have not found any careful study of the pathological changes incident to poisoning by ingestion compared with those in which the mercury entered the blood stream directly.

An exhaustive study of the renal changes in human beings due to ingestion of mercury has been made by Heineke.<sup>1</sup> MacNider<sup>2</sup> in a series of studies has observed the action of mercury in experimental animals.

A search of the literature has failed to reveal a report of mercury poisoning by the intravenous route in a human being. The almost simultaneous death of four persons following the intravenous injection of massive doses of mercuric chloride has afforded the opportunity to compare the effects of the poison thus administered with the effects when taken by mouth. The four persons received intravenous injections of mercury at the hands of a now defunct institution advertised as a "blood serum clinic." Detectives who arrived at the "clinic" were too late to prevent the destruction of records and of most of the medicaments. Several unbroken ampules labeled 1/12 gr. which assayed 5.386 gr. of mercuric chloride were recovered from the waste can. Ampules of other substances such as normal saline and sodium iodide were also found. Since some of the patients with symptoms of poisoning recovered, although all of those interviewed had received more than one injection, it is supposed that mercury was not given each time but alternated with the injection of other substances. The overdose was attributed to a mistake made

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by the chemist who compounded the preparations. It is certain that in each of the four cases here reported one dose of between 5 and 6 gr. of mercuric chloride was given intravenously.

In 1918 Sansum<sup>3</sup> determined the minimal fatal dose of mercuric chloride by intravenous administration in the dog to be quite uniformly 4 mg. per kilogram of body weight. He found larger doses (5 mg. per kilo) necessary to produce anuria, and when animals were once anuric, all attempts to reestablish the flow were futile. Menten<sup>4</sup> found cloudy swelling in the liver and kidneys of rabbits as soon as five minutes after the intravenous injection of as small amounts as 0.002 mg. According to the lowest calculation our cases received at least 6 mg. per kilogram of body weight.

**CASE I \* Clinical History:** M. J., a white male, age 40, was admitted to Cleveland City Hospital on October 19, 1927, complaining of bloody expectoration and difficulty in breathing. Only an incomplete history was obtainable. He had had frequency and dysuria for six years. Late in the summer he had started taking "treatments" at the "blood serum clinic," consisting of intravenous injections. His condition improved, he thought. Three weeks prior to his admission the patient became ill with chills and fever, and later a peritonsillar abscess developed. The only symptom referable to his injections was the onset of anuria two days before his entrance to the hospital. Early on the day of admission the patient was very dyspneic and expectorated bloody sputum. Nothing could be learned about the number of injections he had received.

On examination, he was stuporous, dyspneic and his mouth and lips were covered with blood-stained sputum. Respirations were 10 per minute, the pulse thready, heart-beats 90 per minute, and temperature 35° C. The extremities were cold and moist. The teeth were absent, and the buccal mucosa was covered with bright red blood. Heart, lungs and abdomen were reported negative, but satisfactory examination was impossible. There was a palpable fibrous scar in the anterior urethra.

**Laboratory Findings:** The blood showed hemoglobin 85 per cent; red blood cells 6,300,000 per c.mm.; leukocytes 48,500 per c.mm.; blood urea 59.1 mg. per 100 cc.; uric acid 11.4 mg. per 100 cc.; creatinine 15 mg. per 100 cc. No urine was obtainable. Blood Wassermann was four plus.

Death occurred four hours and forty minutes after admission. The duration of the intoxication was not known since the date of his last injection could not be learned. The clinical diagnosis was acute nephritis, chronic pyelonephritis, urethral obstruction, uremia, with death due to renal insufficiency.

**Autopsy:** The heart weighed 300 gm. and was pale but showed no lesions. There was a confluent bronchopneumonia of both lower lobes. The liver weighed 2200 gm. and showed only cloudy swelling grossly. The spleen weighed 175 gm. and appeared hyperplastic.

\* For permission to report Cases I and II, I am indebted to Dr. R. W. Scott and Dr. O. Saphir of the Cleveland City Hospital.

The left kidney weighed 300 gm. and was large, soft, pale and friable. The boundaries between cortex and medulla were indistinct. Many small abscesses were seen throughout the kidney substance. The pelvis was dilated. The right kidney weighed 100 gm. and showed the same focal abscesses. The organ was pale, and the pyramids smaller and paler than those of the left kidney. There was a reddish gray mucoid exudate in the bladder near the trigone. The walls were thickened and trabeculated. A fibrous stricture was present in the membranous urethra. Distributed through the entire colon there were numerous circumscribed, reddish gray ulcers averaging 1 cm. in diameter. Qualitative tests for mercury on portions of the kidney and colonic contents were positive.

*Histological Examination:* Extensive bronchopneumonia, acute purulent bronchitis, and cloudy swelling of the myocardium were present. There was diffuse and uniform fine granularity and swelling of the liver cells throughout the lobules. Some of the liver cells showed dark brown pigmentation and the sinusoids were moderately engorged with red blood cells. The nuclei of the liver cells were irregular in size, vesicular, and varied greatly in their chromatin content. No evidence of necrosis or regeneration could be seen. In the colon there was edema, necrosis and desquamation of the mucosal epithelium. The gland spaces were represented by an acidophilic debris, with cell outline not discernible. The nuclei appeared fairly intact in the places where the epithelium was not desquamated. There was a moderate degree of polymorphonuclear cell infiltration of the mucosa. The submucosa was edematous and hyperemic.

Examination of the kidney revealed degeneration and necrosis in all parts of the tubular system except the glomerular capsules. This was most severe in the convoluted tubules, both proximal and distal, with slightly less involvement of the loops of Henle and collecting tubules. Corresponding anatomical portions of different tubules in the same kidney varied in the degree of their damage.

There were two types of epithelial cell necrosis. The first was a coagulation necrosis characterized by small, sharply outlined cells having a homogeneous, basophilic cytoplasm. The nuclei were pyknotic or absent. This type occurred almost exclusively in the convoluted tubules. The second type was characterized by large, acidophilic, granular swollen cells which in the more advanced stages of their destruction had indefinite outlines, with ragged inner borders

and large, vesicular or fragmented nuclei, (Fig. 1). This type of necrosis predominated and occurred in all tubules. Not all the necrotic epithelium had desquamated, although in many of the tubules the basement membrane was entirely denuded. Many of the tubular luminae contained coagulated, swollen, disintegrating necrotic epithelial cells. Others contained apparently living cells with clear, distinct, non-granular cytoplasm and intact nuclei. Tubules lined with epithelium showing the second type of necrosis often contained acidophilic vesicular, granular debris. This was canalized and formed bridges connecting the adherent layers of disintegrating cells. There were occasional acidophilic hyaline as well as granular casts. Many of the tubules were filled with red blood cells, and many others with polymorphonuclear leukocytes.

Calcification occurred only in tubules the seat of the first, or coagulation type of necrosis, and was of only slight degree. Occasional calcified or partially calcified cells were seen in the lumina; others were still attached to thin basement membranes.

Regenerated epithelium appeared as low flattened cells with clear cytoplasm and distinct outlines. The nuclei were intact and hyperchromatic. In tubules where regeneration was slight, probably early, the nuclei appeared elongated. Occasional small mitotic figures were observed. Some of the tubules were completely lined with regenerated cells, and others showed beginning regeneration beneath the necrotic epithelium, so that there were two or three layers of cells. Some tubules were so completely filled with regenerated cells that the lumen was occluded.

Many of the glomeruli were large, hypertrophic and cellular, while others were atrophic and hyalinized. The capsules were thickened, crescentic, and many were adherent to the glomerular tufts.

The interstitial tissue was edematous, and showed areas of diffuse polymorphonuclear cell infiltration, as well as localized abscesses containing the same type of cells. There was some interstitial hemorrhage. No relationship between the infiltrating cells and the vascular supply was observed.

Many of the small blood vessels were thick-walled, and some were completely obliterated by a proliferation of the intimal layer. The large vessels showed no changes.

The diagnosis was mercury poisoning, acute nephrosis and pyelonephritis superimposed on chronic glomerulonephritis and arteriolar

nephrosclerosis; confluent bronchopneumonia of both lower lobes; parenchymatous degeneration of the liver; hemorrhagic colitis; cystitis; stricture of the membranous urethra.

**CASE II. Clinical History:** E. S., a white female 35 years of age, was admitted to the Cleveland City Hospital on October 18, 1927. The patient's complaints were sore mouth, vomiting, and inability to urinate. In June the patient went to the "blood serum clinic" in question, and was told she had a four plus Wassermann, for which she took a course of treatments at the "clinic," and had received a total of twenty-five injections during the summer and autumn. The last one was received five days before admission. On the day preceding her last injection she had noticed that her urine was red and within an hour after receiving her last injection there was dizziness, nausea and dryness of the mouth. This was soon followed by much vomiting. Prior to her admission to the hospital she had a few small bowel movements which did not appear bloody. She passed no urine for five days prior to her admission on October 18, and none was obtained by catheterization. She had a history of a secondary eruption seven years ago, and had been treated with injections and mercury rubs. There had been numerous miscarriages since that time, all of them being at about two months.

Examination revealed a rational patient, vomiting considerably. The mouth was sore, lips were dry, and tongue was coated, with many small ulcers on the under surface. The teeth were loose, there was a marked gingivitis and the submaxillary glands were swollen and tender. The abdomen was distended and tympanitic, but no tenderness, or masses were revealed. The remainder of the examination was negative. Respirations were 20, pulse 80 to 90 and blood pressure systolic 132 and diastolic 84.

**Laboratory Findings:** No specimen of urine was obtained until the fourth hospital day. The first one was small in amount and contained gross blood. The second was clear, alkaline, and had a specific gravity of 1.008. It contained a small amount of albumen and sugar and numerous red blood cells and leukocytes but no casts. On the following day it was cloudy, alkaline, with a specific gravity of 1.012 and gave a positive test for albumen but was negative for sugar. Microscopic examination showed hyaline casts, many red blood cells and leukocytes. On admission she had a leukocyte count of 13,300, red blood cell count of 3,990,000 and 75 per cent hemoglobin. Chemical analysis of the blood on October 20 showed urea 237 mg. per 100 cc., creatinine 6.3 mg. per 100 cc., uric acid 5.3 mg. per 100 cc., chlorides 412 mg. per 100 cc.

The patient received sodium thiosulphate intravenously, saline infusions, and high colonic irrigations. Her course was a febrile throughout. She died at 10:45 P.M. on October 24, six days after admission and eleven days after her last injection.

**Autopsy:** Performed at the morgue by the county coroner, Dr. Hammond, and to him I owe my thanks for permission to use his findings. The right arm and forearm were swollen and discolored on the volar surface, about the region of the median basilic vein. The heart was negative, but the lungs showed a moderate amount of edema and congestion. The report stated that there was fatty

degeneration throughout the liver. The kidneys were large and together weighed 470 gm. They were pale both externally and on section. The glomerular markings were visible, and there were radial striae in the cortex. The boundary between cortex and medulla was indistinct. The spleen was large, weighing 180 gm. It was dark red in color, soft in consistency, and the follicular markings were not obscured.

A quantitative determination for mercury showed none in 100 cc. of blood, none in a portion of kidney, and 3.5 mg. in a 15 gm. section removed from the wall of the large bowel.

*Histological Examination:* There were no abnormalities of the myocardium and the lungs showed a slight amount of congestion and edema. The sinusoids of the spleen were engorged with blood. The liver cells were moderately swollen and granular, all portions of the lobule being uniformly involved.

Sections of the colonic wall showed no change other than what appeared to be autolysis of the mucosa.

Sections of the kidneys showed diffuse degeneration of the epithelium in all portions of the tubular system except the glomerular capsules. The tubules were distended, and the loops of Henle and collecting tubules showed more damage than did those in the preceding case.

The first type of necrosis, the coagulation type, predominated, and was as severe in the loops of Henle as in the convoluted tubules. Necrotic cells with swollen, acidophilic, granular cytoplasm and vesicular nuclei were seen but were not conspicuous. In many tubules desquamation was complete, leaving a denuded basement membrane. Others were partially or completely lined by necrotic epithelium.

Casts were few and consisted predominantly of epithelial cells which had undergone coagulation necrosis. Hyaline casts were seen. Some of the most widely dilated tubules were filled with an acidophilic, granular, vesicular substance.

There was considerable calcification of the necrotic epithelium. This was observed in that which was still attached to the tubular wall, as well as in masses of completely desquamated cells. It occurred only in those cells showing the coagulation type of necrosis.

Regenerated epithelial cells were present but not abundant. Where they occurred, they undermined necrotic cells, but the tubules were not as completely filled as in the preceding case. The regener-

ated cells presented the same details of structure as previously described.

There was much edema, as well as a moderate degree of lymphocytic infiltration of the interstitial tissue, (Fig. 4).

The glomeruli and blood vessels showed no changes.

The diagnosis was acute nephrosis; parenchymatous degeneration of the liver; pulmonary edema; passive hypermia of the lungs and spleen.

*CASE III.\* Clinical History:* J. K., a white male, aged 49 years, was admitted to the Cleveland Clinic Hospital on October 21, 1927, with a history of anuria for one week. The patient had a sudden chill eight days previously while riding home on the street car from a visit to the "blood serum clinic," where he had received an intravenous injection. On reaching home his temperature was 102° F. There was severe nausea, emesis, and pain in the abdomen. The following day he had diarrhea with bloody and mucous stools, and stopped passing urine. After three days of these manifestations he felt better for a day and then became worse. By this time a marked stomatitis had appeared. Nausea and vomiting persisted. The stools were frequent (as often as every hour on some days) and continued to be bloody.

On admission his temperature was 36.8° C, pulse 86, and blood pressure systolic 130 and diastolic 70. Examination showed a well developed and nourished male with marked herpes about the lips, stomatitis, foul, coated tongue and uriferous breath. There was tenderness over the entire abdomen, with a slight degree of spasticity. There was bleeding from the rectum.

*Laboratory Findings:* The blood showed 4,120,000 red blood cells, 85 per cent hemoglobin, and 17,300 leukocytes. A differential count revealed 90 per cent polymorphonuclear leukocytes and 10 per cent lymphocytes. There were 312 mg. of urea, 182 mg. of sugar, 6.6 mg. of uric acid and 465 mg. of chlorides per 100 cc. of blood. The blood Wassermann was four plus and the Kahn test three plus. The stools gave a strongly positive benzidine test, and bright red blood was visible grossly.

Treatment consisted of saline infusions and the intravenous injection of sodium thiosulphate. The patient became irrational on the morning after admission, lapsed into coma, and died in the afternoon. His temperature remained below normal until just before death when it rose to 36.8° C. His pulse varied from 90 to 100 and his respirations from 20 to 25. Anuria persisted during his stay in the hospital. At no time did he have convulsions.

*Autopsy:* The autopsy was performed four hours after death. The gums showed a bluish tinge. There was pitting of the chest wall, but no edema of the extremities. The colon was covered with glistening peritoneum. There was no free fluid in any of the serous cavities. The lungs showed a moderate degree of congestion and

\* I wish here to acknowledge my sincere thanks to Dr. Phillips and Dr. Ball for permission to report this case.

edema. The heart, spleen, stomach, duodenum and jejunum showed no gross changes. Beginning about one meter proximal to the ileocecal valve there was a reddened and congested appearance of the mucosa. There were occasional superficial ulcerations averaging 1 to 2 mm. in diameter in the free margins of the mucosal folds. Such areas were hemorrhagic, with small blood clots attached to the ulcers. They were irregular in distribution. The mucosa of the cecum and colon throughout its extent showed irregularly shaped and distributed superficial ulcerations averaging 8 by 3 mm. and having attached blood clots. The intervening mucosa was dark red. The colon was filled with a bright red, thick fluid.

The liver weighed 1450 gm., was smooth, dark brown in color, and had slightly rounded margins. The cut surface showed a normal architecture.

The left kidney weighed 170 gm., the right, 165 gm. They were firm, smooth, and showed a yellowish gray cut surface. The cortex was 7 mm. thick and well differentiated with pale yellowish radial markings.

Pelves, ureters, bladder, prostate and adrenals all grossly appeared normal.

Small portions of the liver (20 gm.), colon (10 gm.) and kidney (10 gm.) gave strongly positive qualitative tests for mercury. Electrolytic quantitative tests showed 10 mg. of mercury in 15.5 gm. of colonic contents.

*Histological Examination:* There was slight cloudy swelling of the heart muscle cells. The lungs showed a very early bronchopneumonia and considerable congestion and edema. Passive hyperemia of the spleen was found. The gastric mucosa showed no change other than autolysis, and the ileum was unchanged. There was coagulation necrosis of the colon mucosa, with diffuse and focal areas of polymorphonuclear leukocytic infiltration of the mucosa and submucosa. In addition, the submucosa showed much congestion and hemorrhage. The liver cell cytoplasm was diffusely swollen and granular. The blood vessels were filled with red blood cells but the sinusoids were empty. There was swelling, vacuolization and granularity of the adrenal cells, diffuse in the medulla, but only occasional cortical cells were involved.

The tubular damage in the kidneys of this case was less extensive and severe. Only the convoluted tubule epithelium showed necrosis,

while the collecting tubules and loops of Henle were the seat of moderately severe parenchymatous degeneration.

Both types of necrosis were observed, with a slight predominance of the coagulation variety. Much of the necrotic epithelium was desquamated, and some detached epithelium which appeared to be living was observed. In the loops of Henle and collecting tubules the cytoplasm was swollen, finely granular and showed some fat vacuolization. The nuclei were intact. There was little desquamation of the epithelium in these tubules.

All tubules contained many hyaline casts in addition to masses of desquamated epithelium. No red or white blood cells were seen in the lumina.

There was much calcification of the necrotic cells. It was seen in desquamated epithelium as well as in that which was still attached (Fig. 3).

Regenerated epithelial cells occurred as a thin layer of flattened epithelium on the basement membrane, with intact nuclei and cytoplasm. Occasional mitotic figures were seen. A few tubules were found where the regenerated layer of cells was overlaid with necrotic, incompletely desquamated cells.

Interstitial edema was present but not so pronounced as in the two preceding cases. A few focal areas of lymphocytic infiltration of the interstitial tissue were present in the cortex.

Except for an occasional hyalinized glomerulus, the glomeruli showed no changes.

The medullary vessels were engorged with red blood cells, but there were no changes in the vessel walls.

The diagnosis was acute nephrosis; hemorrhagic colitis; early bronchopneumonia; parenchymatous degeneration of the liver; pulmonary edema and congestion; cloudy swelling of the myocardium.

**CASE IV.\*** *Clinical History:* C. V. B., a white male 60 years of age was admitted to Lakeside Hospital on October 22. The patient gave a history of having had three intravenous injections within five days. These were to constitute a "course" to cure "neuritis" in his left shoulder and to lower his blood pressure. The patient was not a luetic. After having his second injection he thought he did not feel as well as before, and after his third injection he became dizzy and weak. He was confined to his bed and finally referred to the hospital by his private physician. There had been no anuria, hematuria or bloody stools.

On admission the patient was rational and talkative. There was blood-

\* I am indebted to Dr. M. A. Blankenhorn for permission to report this case.

stained sputum in his mouth, and his tongue and mucous membranes were dry. The gums were swollen, soft, spongy, dark, and showed minute hemorrhagic spots. The abdomen was distended and tympanitic. There was no edema.

After two days his condition seemed improved. He had been passing fairly copious quantities of urine of low specific gravity (1.010) which did not vary. There was a moderate amount of albumen. Pus cells were numerous and a few hyaline casts were seen. The leukocyte count was 12,000. The red cells and hemoglobin were within normal limits. His blood pressure was systolic 180 and diastolic 90. Phenolsulphonephthalein tests were done on successive days (fourth and fifth) with no excretion of the dye in two hours. On the fourth day his blood urea was 187 mg. Toward the latter part of his stay in the hospital he had incontinence of urine and feces. His stools were tarry, and later stained with fresh blood. Throughout his stay his respirations were slow, and his temperature was slightly elevated until the last day, when it became subnormal. On the last day at 10 A.M. he developed sudden pulmonary edema, foamy material coming from his mouth and nostrils. This cleared up after 1/100 gr. of atropine was given hypodermically, but the patient died eight hours later, on the eighth hospital day.

*Autopsy:* The postmortem examination was performed about three hours after death. No edema was observed. The neck veins were engorged. The gums were swollen, soft and spongy, showing a few small hemorrhagic areas. The buccal mucosa was hyperemic. No free fluid was encountered in any of the serous cavities. The stomach and colon were dilated. There was some catarrhal exudate in the lumen of the trachea and bronchi. The heart showed no abnormalities. A few small yellowish plaques of intimal thickening were encountered in the arch and ascending portions of the aorta. The liver weighed 1575 gm. and was fairly firm in consistency. It was pale, and on cut section the architecture was somewhat obscured. The spleen weighed 180 gm., was firm in consistency, and showed considerable passive hyperemia. The kidneys weighed together 500 gm. The capsule stripped readily, leaving a pale red, smooth surface. The cortex was pale and slightly thickened but showed radial striations. The pyramids were engorged with blood, and presented a marked contrast to the pale, swollen appearance of the cortex. The bladder was thick-walled and trabeculated. The prostate showed a diffuse nodular enlargement. The esophagus, stomach, and small intestines showed no changes. Beginning at the cecum and extending throughout the length of the colon, the mucosa was hyperemic, edematous, and showed minute hemorrhagic areas with no ulceration or necrosis. The brain showed no changes but there was edema of the leptomeninges.

A quantitative determination gave 11.09 mg. of mercury in one entire kidney.

*Histological Examination:* Sections of the lungs showed some of the bronchi to be partially and others completely filled with an exudate containing polymorphonuclear leukocytes. The bronchial walls showed no change, however, and the alveolar walls and septa appeared normal. In the liver there was much fatty vacuolization of the cells in the central areas of the lobule, extending about halfway to the peripheral zone. There was granularity, vacuolization, and variation in chromatin content of the nuclei. In the peripheral zones the cells were swollen and granular, but the nuclei appeared undamaged, and there was less vacuolization of the cytoplasm. The splenic vessels and sinusoids were engorged with blood. Sections of the colon showed necrotic epithelium of the mucosa with a hemorrhagic stroma and edematous submucosa. The medullary portions of the adrenal showed swollen and vacuolated cells with granular cytoplasm. The cortical cells appeared unchanged. The prostate was hypertrophic.

In this case the tubular epithelial cell degeneration and necrosis in the kidneys were less pronounced than in any of the others. The convoluted tubules were the most severely damaged, the collecting tubules showing no damage other than moderate cloudy swelling. In the loops of Henle there was a degree of damage intermediate between that of the convoluted tubules and the collecting tubules.

Necrosis of both types of about equal extent was seen, but there was relatively little desquamation.

Masses of necrotic, desquamated epithelial cells, hyaline casts, red blood cells, and white blood cells were all seen within the lumina of the tubules. Calcification was not present.

Regeneration of the tubular epithelium was the prominent feature. Some tubules were lined with flat, newly formed epithelium similar in appearance to that in the preceding cases. Part of these were overlaid with irregular layers of necrotic epithelium. Others were completely lined with a single layer of the newly formed cells. A conspicuous feature, however, was the occurrence of newly formed epithelial cells, rich in cytoplasm, having hyperchromatic nuclei, and frequently showing a piling up of the cells two and three layers deep. Mitoses were frequent, (Fig. 2).

The glomeruli showed no changes.

In the interstitial tissue there was a moderate degree of edema. Patchy areas of lymphocytic and polymorphonuclear cell infiltration were seen in the regions of tubules containing white blood cells. There were no vascular changes.

The diagnosis was mercury poisoning; acute nephrosis; hemorrhagic colitis; parenchymatous degeneration of the liver; pulmonary edema; congestion of the lungs and spleen; hypertrophy of the prostate.

#### DISCUSSION

Degeneration and necrosis were observed in the tubular epithelium of all four of these cases. The epithelial damage ranged from parenchymatous degeneration to necrosis with calcification. The most severe lesions were seen in the proximal and distal convoluted tubules, while the loops of Henle and the collecting tubules were involved to a lesser degree. In Cases I and II there was marked degeneration and necrosis present in all parts of the renal tubular system, except for the glomerular capsule, while in Cases III and IV the injury to the loops of Henle and the collecting tubules was manifested only by parenchymatous degeneration. In only one of the cases (Case III) was there any considerable fatty degeneration of the tubular epithelium.

Two types of cell death were seen. Simple coagulation necrosis of epithelium was the more common, the cell outline being left intact with pyknosis or disappearance of the nucleus. Such cells were seen still attached to the basement membrane as well as free in the lumen. Frequently, however, the cell death was manifested by a marked swelling, the inner margin being ragged and the nucleus large, vesicular, and fragmented. In tubules whose epithelium was the seat of this type of necrosis, the lumen was often filled with a vesicular acidophilic granular material resembling the degenerated cytoplasm which occasionally bridged the tubule from one side to the other. These changes were not constant in their location even in the same kidney but all the kidneys examined showed a co-existence of both types of necrosis.

The presence together of both types of cell death is of interest in connection with MacNider's<sup>2</sup> findings in a study of the renal injury of dogs following mercuric chloride poisoning. He concluded that the primary nephrotoxic action of mercury is predominantly on the tubular epithelium and consists of diffuse coagulation necrosis with-

out edema, and that the swelling and necrosis of epithelium with fragmentation of nuclei are associated with the acid intoxication and anuria which appear later. The latter type of necrosis developed as late as the ninth day of intoxication when the urine no longer contained mercury. The presence of diffuse coagulation necrosis in the kidneys of our cases as late as the twelfth day of the intoxication indicative, according to MacNider, of the nephrotoxic action of mercury would imply a very delayed excretion of the poison. This indicates that a large part of the intravenous doses of mercury was fixed and slowly liberated.

Tubular casts of many kinds were seen. Pus cell casts were present in Cases I and IV, while red blood cells were present in the tubules of Cases I, II and IV. Cellular casts of coagulated necrotic epithelium as well as disintegrated swollen necrotic cells were seen in all four cases. In addition there were in all cases, rounded, apparently living epithelial cells with intact, normal appearing nuclei free in the tubules. Hyaline and granular casts were present in all cases, the latter being often vesicular and associated with necrosis in the lining epithelium.

Calcification of necrotic epithelium was present in the first three cases and was more marked in Case III. The kidneys of this case likewise showed the most extensive coagulation necrosis of tubular epithelium. No calcification of apparently living cells was seen and no evidence was found to support Leutert's <sup>5</sup> contention that calcium deposition occurs in injured but still functioning cells. The necrotic cells showing calcium deposition occurred in masses free in the lumen of the tubules as well as in their original places in the lining of the tubules. These calcified cells were more abundant in the cortex but were occasionally seen in Henle's loops or even the collecting tubules.

Regeneration of the tubular epithelium was seen in all four cases and was most extensive in Case IV in which tubular damage and calcification were least manifest. As described by Heineke <sup>1</sup> the earliest stage of regeneration consisted in the formation of a flattened layer of young cells between the necrotic epithelium and the basement membrane of the tubules. He ascribed the desquamation of the degenerated and necrotic cells to this proliferation but we observed occasional tubules completely denuded of their epithelium with no evidence of epithelial regeneration. In Case IV the epithelial regeneration was quite exuberant, many of the tubules being almost

occluded by the young cells with a large amount of cytoplasm and nuclei of varying size and chromatin content. Small mitotic figures were observed and in places the new epithelium was piled up several layers deep. The contrast between the young and the swollen degenerated old epithelium was sharp because of the definite cellular outline, the acidophilic, clear cytoplasm and the absence of vesicular and fragmented nuclei in the regenerated epithelium. No evidence of new tubule formation was observed. The most pronounced epithelial regeneration occurred in the convoluted tubules which were likewise the seat of the most severe degeneration. The glomeruli were hyperemic but there was no other change except for the chronic diffuse glomerulonephritis seen in Case I and the scattered hyalinized glomeruli seen in Case III.

The interstitial tissue was edematous in the four cases with patchy lymphocytic infiltration. Case I was complicated by pyelonephritis and showed abscesses throughout the cortex with extensive peripheral zones of leukocytic infiltration. In Case IV there was interstitial leukocytic infiltration around some of the pus-containing tubules. Except for the complicating arteriosclerosis in Case I there was no evidence of vascular change. Neither the thrombosis with multiple infarction observed by Kaufmann<sup>6</sup> nor the perivascular infiltration reported by Karvonen<sup>7</sup> occurred in these cases.

Damage to the liver has been noted by many investigators of the pathological changes incident to mercury poisoning. Burmeister and McNally<sup>8</sup> considered the degeneration in the liver to be due to the action of mercury as such during absorption and elimination. They found the liver injury to vary with the duration of the intoxication and the kidney injury to vary with the amount of mercury introduced as well as with the duration of the intoxication. They observed a marked parenchymatous degeneration of the hepatic cells of the central zones. MacNider<sup>2,9</sup> in experimental studies on animals in which the mercury was introduced by stomach tube reported the occurrence of edema and necrosis in the periphery of the liver lobules. Foster<sup>10</sup> called attention to the degeneration of liver cells in a fatal case of mercurial poisoning and Turrettini and Piotrowski<sup>11</sup> described two cases of fatal mercuric poisoning with liver damage so severe that they considered the liver injury to be the cause of death.

We observed a uniformly mild grade of parenchymatous degenera-

tion of the liver in all of our cases with no necrosis and no selective localization to any part of the lobules. This raises the question of whether the severe hepatic damage is due to the enteral route of intoxication and therefore not present in intravenous poisoning.

Epithelial necrosis and ulceration in the colon was a constant finding and all of the cases showed lesions of varying severity in the large bowel. Cases II and IV showed simple epithelial necrosis while the entire colon of Cases I and III was the seat of multiple deep hemorrhagic mucosal ulcers. There was no gastro-enteritis in any of the cases.

#### SUMMARY AND CONCLUSIONS

1. The four cases here reported survived periods of intoxication ranging from six to twelve days following the intravenous injection of mercuric chloride in a dosage of over 5 mg. per kilogram of body weight.

2. Two types of necrosis were found in the kidneys of each of the four cases.

3. The desquamation of renal tubular epithelium was not necessarily dependent upon epithelial regeneration beneath the necrotic cells.

4. Calcification was observed only in epithelium the seat of coagulation necrosis and the calcified cells were seen *in situ* as well as free in the lumina of the tubules.

5. The liver damage in these cases consisted only of a mild parenchymatous degeneration in contrast to the severe hepatic changes observed after mercury poisoning by mouth.

6. Epithelial necrosis and mucosal ulceration of the colon were noted in the four cases.

7. The renal changes due to mercury poisoning in man are essentially the same whether the mercury is administered by mouth or by the intravenous route.

8. Gastro-enteritis does not occur after the intravenous injection of large doses of mercury.

I wish to record my sincere thanks to Dr. Alan R. Moritz, the pathologist in charge at Lakeside Hospital, for his kind assistance and advice in the preparation of this report.

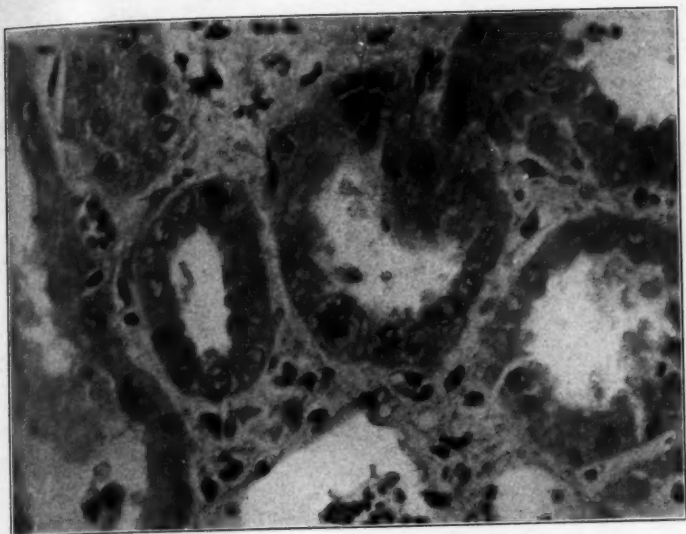
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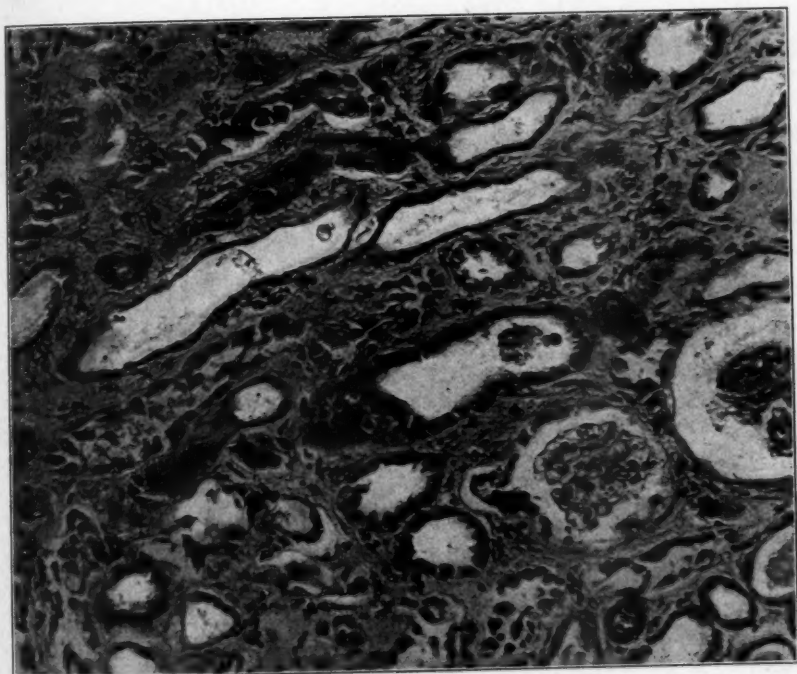
## DESCRIPTION OF PLATES

## PLATE 76

- FIG. 1. Case I. Hematoxylin and eosin stain, showing swollen type of epithelium necrosis and regeneration, with mitotic figure.  $\times 475$ .
- FIG. 2. Case IV. Showing coagulation necrosis of tubular epithelium and piling up of regenerating cells in one of the tubules.  $\times 250$ .



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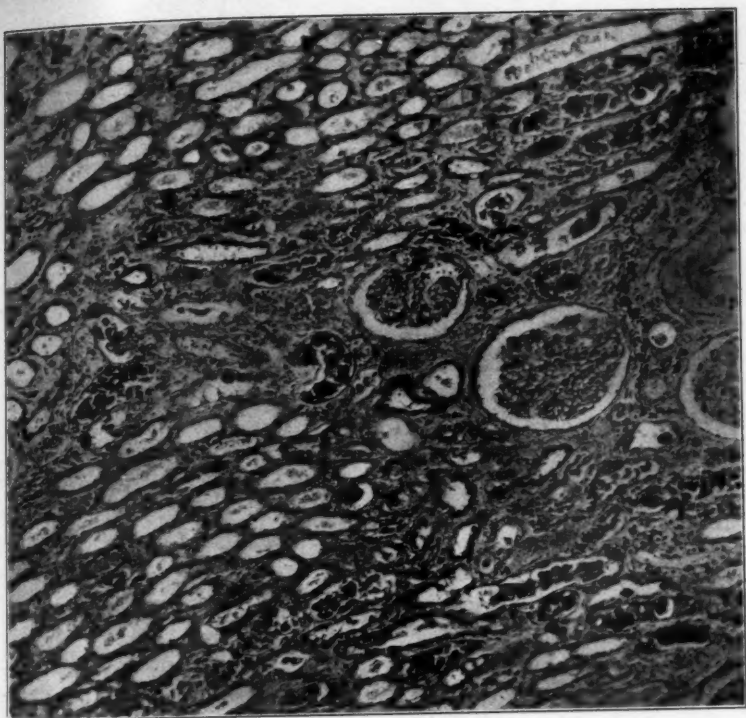
Harmon

Human Mercuric Chloride Poisoning

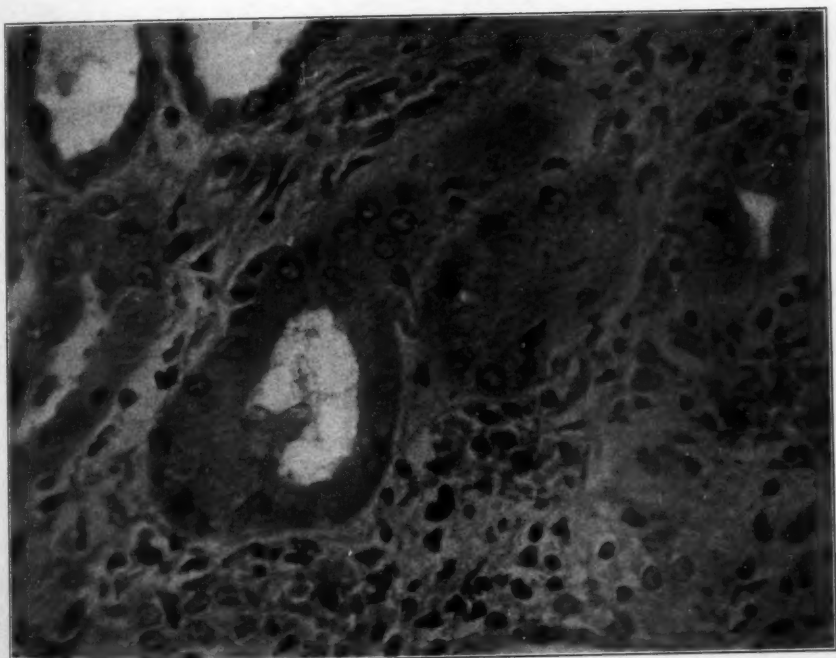
PLATE 77

FIG. 3. Case III. Low power magnification showing coagulation necrosis and calcification of necrotic epithelium.

FIG. 4. Case II. Showing interstitial edema and lymphocytic infiltration. Tubules show swollen type of epithelial necrosis and bridging of lumen by necrotic cytoplasm.



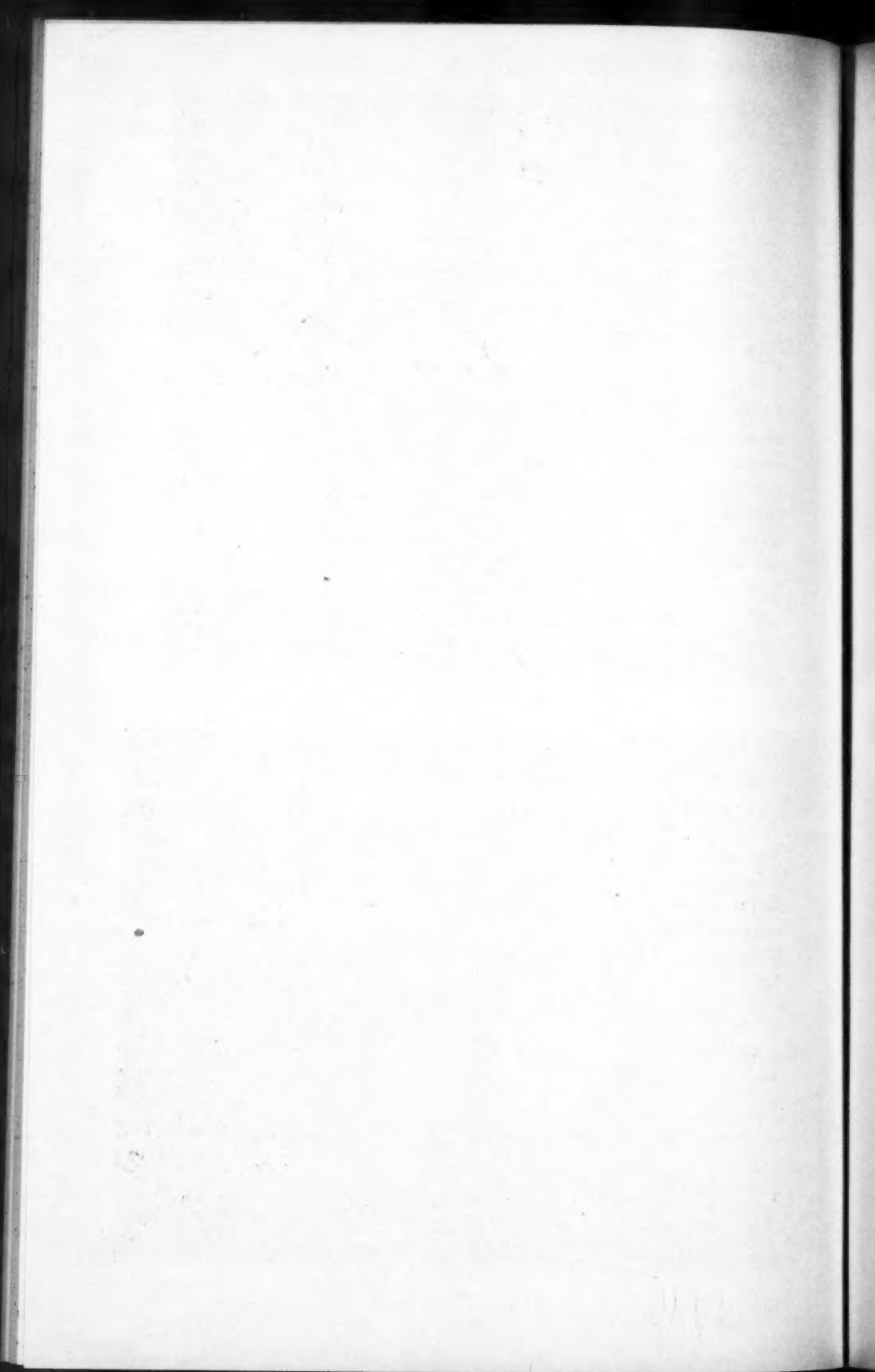
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Harmon

Human Mercuric Chloride Poisoning



## TISSUE CULTURE OF INTRACRANIAL TUMORS

### WITH A NOTE ON THE MENINGIOMAS

FREDERICK E. KREDEL

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Boston, Mass.)

It has been the purpose of this clinic for some time to utilize the tissue culture technique in the study of intracranial tumors. It was thought that observations on living tumor cells cultivated *in vitro* might profitably supplement the usual histological examination of fixed tissue and add not a little to our present knowledge of their histogenesis. This present paper records the results of some preliminary studies in this direction.

### TECHNIQUE

The technique employed is the hanging-drop method of Lewis and Lewis.<sup>1</sup> A bit of healthy tumor tissue secured at operation is cut up in Locke or Tyrode solution into small pieces a millimeter or less in diameter. A piece is placed in the center of a clean sterile coverslip and a drop of heparinized human plasma is added. The coverslip is then inverted and sealed by means of a vaseline ring on a depression slide. In some cases a drop of tumor extract is added to promote growth. Aseptic precautions are observed throughout. The cultures are incubated at 37.5° C.

In successful cultures (Fig. 1) the cells migrate out within twenty-four hours on the under surface of the coverslip. One can then study under the highest powers of the microscope the morphology of the living cells as well as their reaction to vital dyes and to particulate matter.

### TUMORS CULTURED

In order to ascertain what types of tumors would be favorable material for cultivation all tumors removed on the neurological service over a period of several weeks were cultured. A list of these

\* Received for publication May 12, 1928.

together with the percentages of success with each is shown in the table below:

TABLE I  
*Tumors Cultured*

	No. cultured	Satisfactory	Per cent
Meningioma.....	5	3	60
Metastatic carcinoma.....	2	1	50
Spongioblastoma multiforme.....	1	1	100
Pituitary adenoma.....	5	0	0
Astrocytoma.....	3	0	0
Acoustic neuroma.....	2	0	0
Cystic spinal tumor.....	2	0	0
Cysts.....	3	0	0
Unclassified malignant epithelial tumor.....	1	0	0
Total .....	24	5	21

The results were not satisfactory in many of the tumors. With the simple technique employed one could hardly expect the cells of slowly growing tumors like the astrocytomas to migrate actively in tissue culture. In a few of the pituitary adenomas a small number of epithelial cells wandered out from the explanted piece, forming a loose sheet; a growth pattern characteristic of glandular epithelium. In cultures of two cystic tumors of the spinal cord ciliated epithelial cells migrated out.

Satisfactory growth occurred in cultures of tumors of three types: meningioma, metastatic carcinoma, and spongioblastoma multiforme.\* The success with the one specimen of spongioblastoma inspires the hope that the gliomas of relatively undifferentiated cell type may prove to be favorable material for study by tissue culture methods.

#### MENINGIOMAS

Active migration occurred in cultures of three out of five meningiomas. The cultures were kept alive for as long as three weeks by renewing the medium every three or four days and, in a few cases by subculturing the explanted piece. Attempts were made in our observations on these cultures to determine the precise nature of the outwandering cells.

The typical cells that grew out seemed to be of the mononuclear-

\* Since Mr. Kredel made this preliminary study in the summer of 1927 we have had success with other types, notably with the acoustic neurinomas. H. C.

macrophage series rather than fibroblasts. Most of the cells were ameboid in shape. While some of them were pyriform and unipolar, the multipolar stellate form with many cell processes characteristic of fibroblasts was not in evidence during the first few days after explantation. Some cells showed a rosette of neutral red granules in the region of the centrosphere. In older cultures, however, the cells appeared gradually to assume a form more closely resembling that of fibroblasts.

To check these morphological observations with some physiological criterion the phagocytic power of the outwandering cells was tested by adding to the cultures a suspension of finely divided carmine in Locke solution. In cultures of a few days the cells ingested large numbers of carmine particles. Fig. 2 is from a seven-day culture of meningioma. This photograph illustrates both the characteristic morphology and the remarkable phagocytosis of carmine noted in the younger cultures.

After cultivation for a week or more the cells seemed to lose their phagocytic ability. Fig. 3 shows a group of cells in an eleven-day culture from the same tumor as the cells shown in Fig. 2. These latter cells did not phagocytize carmine to any noticeable extent. An anomalous result was obtained with a twenty-day culture, a field of which is shown in Fig. 4. The cells failed to ingest any carmine during the first half hour after the suspension was added. But at the end of four hours the cytoplasm had become tremendously swollen, distorted, and contained many carmine particles. We are inclined to believe that this last was an artifact due to the basicity of a poorly prepared carmine suspension, the reaction of which was found to be pH 7.9.

These observations, although suggestive, do not add much of critical importance to our present knowledge of the histogenesis of the meningiomas. If the view of Mallory<sup>2</sup> and Penfield<sup>3</sup> is correct that the type cell of these tumors is the fibroblast, the outwandering cells in our cultures must be clasmotocytes present in the stroma. This possibility must be considered, for Lewis and Gey<sup>4</sup> have shown that clasmotocytes migrate out abundantly in cultures of mouse sarcoma and have pointed out that the presence of large numbers of such phagocytes may well be overlooked in fixed sections. On the other hand, these observations conflict in no way with the view of Cushing<sup>5,6</sup> that the histogenesis of the meningiomas is explained

on the basis of tumefaction of clusters of meningocytes that line the arachnoid villi and which possess phagocytic powers. The meningiomas when examined fresh by supravital technique are found to contain large numbers of these phagocytic cells and in his opinion they presumably represent a constituent part of the tumor.

#### SUMMARY

Successful tissue cultures with the hanging-drop technique have been made from meningioma, metastatic carcinoma, and spongioblastoma multiforme.

Phagocytic cells migrated out in large numbers in meningioma cultures. The ability of these cells to ingest particulate carmine decreased progressively with the age of the cultures.

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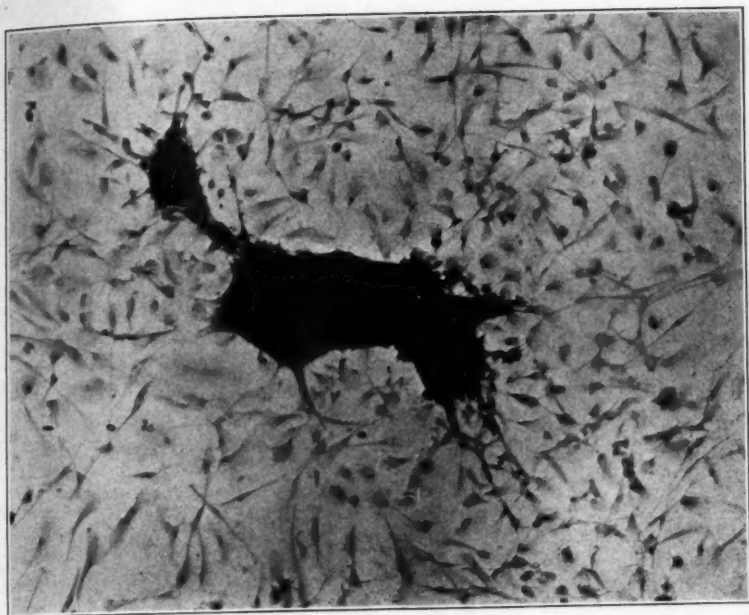
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#### DESCRIPTION OF PLATES

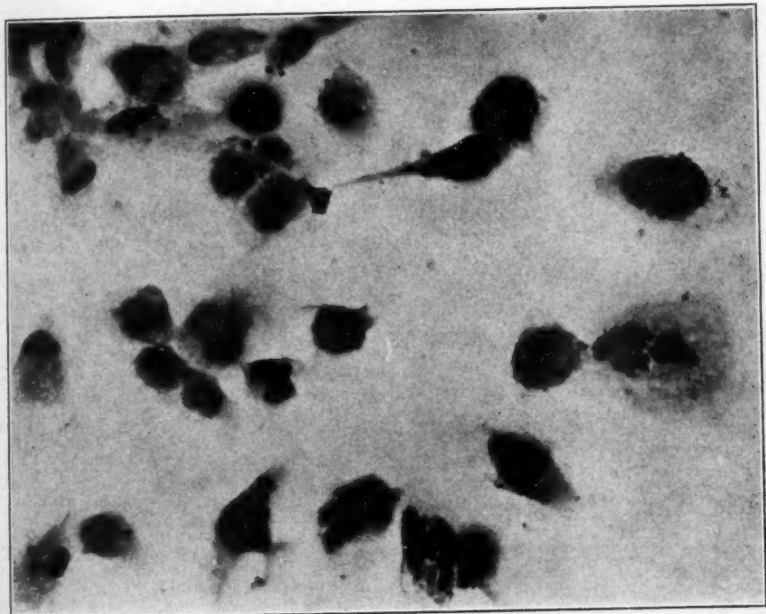
##### PLATE 78

FIG. 1. An eight-day culture of a metastatic carcinoma (fixed in formalin, H. and E stain), showing an abundant outgrowth of multipolar and often multinuclear cells containing large granules not taking neutral red.  $\times 100$ .

FIG. 2. A typical field of growing meningioma (seven-day culture, H and E stain). Most of the cells have ingested large numbers of carmine particles and are obviously phagocytic.  $\times 600$ .



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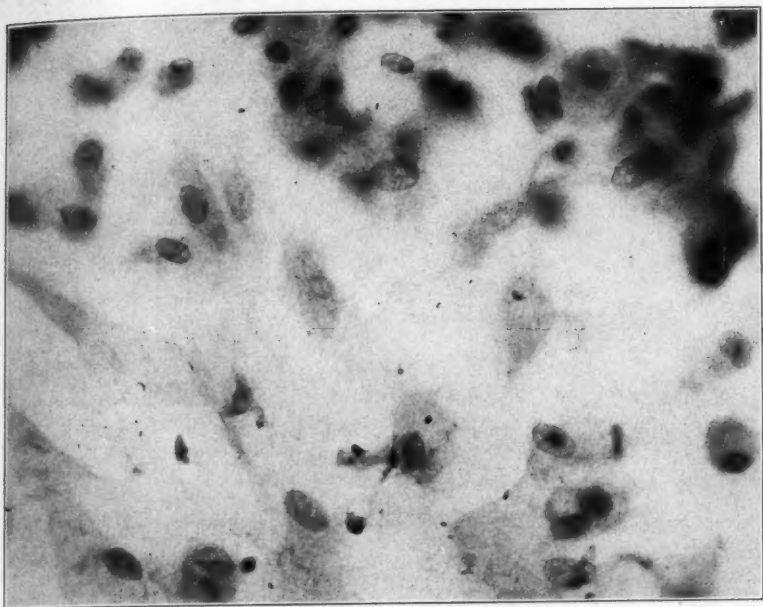
Kredel

Tissue Culture of Intracranial Tumors

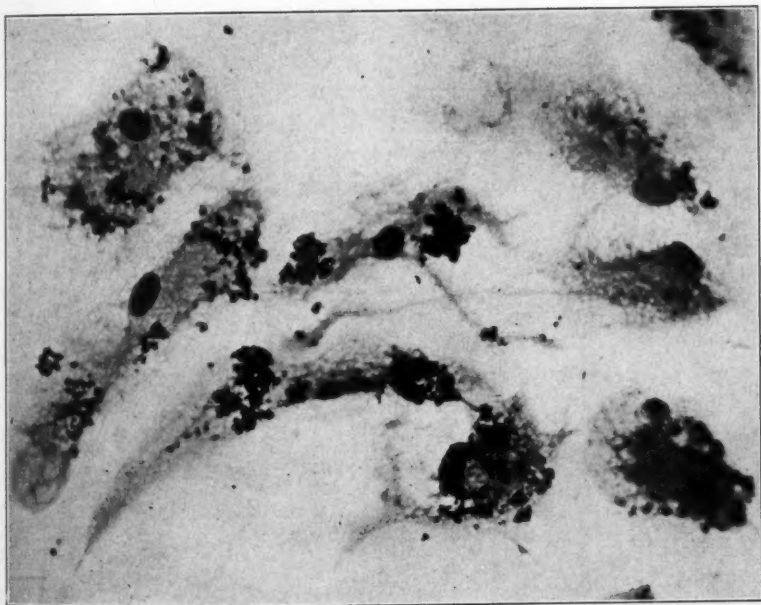
PLATE 79

FIG. 3. An eleven-day culture of same tumor as Fig. 2 (H and E stain), showing cells (fibroblasts or meningocytes?) which do not ingest carmine.  $\times 600$ .

FIG. 4. Cells from same tumor as Fig. 2 after twenty-day growth (H and E stain), showing them richly laden with carmine particles.  $\times 600$ .



3



4

Kredel

Tissue Culture of Intracranial Tumors



A STUDY OF THE TISSUE CHANGES IN EXPERIMENTAL  
BLACK TONGUE OF DOGS  
COMPARED WITH SIMILAR CHANGES IN PELLAGRA\*

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INTRODUCTION

In the present communication there is presented a report on the gross and microscopic changes in a graded series of early and late cases of experimental black tongue of dogs. This study was made in connection with, but essentially independently of, the study of experimental black tongue being conducted at the Hygienic Laboratory by Goldberger, Wheeler, Lillie and Rogers.<sup>1</sup> The material was secured from dogs that had served as test animals in certain of the feeding experiments carried out by Goldberger and associates in the course of their study of black tongue. These workers furnished the summary statement, presented in the appendix, of the significant points in the history of each of the animals from which tissues were secured.

*Technic:* The animals were killed with illuminating gas and careful autopsies were done immediately after death. Tissues for histologic study were fixed in a modified Zenker's fluid (that is, Zenker's without acetic acid) and stained with Giemsa's stain. Formol fixation and hematoxylin-eosin staining were also used in some cases. Weigert preparations were made on sections of the spinal cord in the later cases of the series. Every important organ and tissue was examined histologically in each case.

GROSS PATHOLOGY

Care was taken not to confuse the lesions of the disease under consideration with the effects of partial starvation which at times complicated it. The most typical and important lesions appeared in dogs still in good states of nutrition. The effects of partial starvation were seen in three animals killed late in the series. They will

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not be described as they are not pertinent to the condition under consideration.

*Mouth:* The earliest recognizable lesion is a reddening of some part of the oral mucosa, which is much more apparent during life than after death. This reddening usually begins in the floor of the mouth or the cheeks, or along the inner side of the upper lip. Lesions of greater severity show a more marked reddening and in addition raised areas of variable size and shape. The raised areas are either darker red than the surrounding tissue or of a greenish gray color. This appearance is due to superficial necrosis of the epithelium and the formation of a pseudomembrane on the surface, and for this reason is looked upon as a complicating lesion.

When the disease nears its natural termination the whole surface of the mouth and pharynx becomes deep red, obviously swollen and granular with greenish or grayish discolored areas. In dogs killed in the agonal stage of the disease the oral and pharyngeal mucosae are affected in their entirety and the lesion may extend into the esophagus. At this stage diffuse reddening is apparent in some areas but the major share of the surface is covered with gray or green pseudomembrane.

In dogs left to die there is found at postmortem an extensive superficial necrotic and diphtheritic condition of the upper alimentary tract.

*Skin:* Lesions of the skin of the scrotum were present in four dogs of this series. As seven of the thirteen dogs were males, and as similar lesions were observed in other dogs suffering from the disease experimentally induced by Goldberger and associates, but not studied in this series, the scrotal changes are not looked upon as accidental. The appearance of the scrotal lesion has been described by Goldberger and Wheeler;<sup>2</sup> it contrasts markedly with the normal, smooth, pale surrounding skin. No general cutaneous changes were observed in any of the dogs. No unusual tendency to shedding was apparent.

*Intestines:* Intestinal lesions were not observed in the earlier cases but in three later specimens the colon was thinner than normal, the inner surface was stained with reddish brown mucus and the mucous membrane generally was reddened.

*Other Organs:* No gross changes referable to the disease were observed in other tissues or organs. The bones were carefully examined.

Particular attention was paid to the periosteum of the long bones. It was uniformly of normal color and thickness. Periosteal hemorrhages were not seen.

The brain and cord were examined in each case but no gross changes were observed.

The characteristic gross appearances of the disease have been confined to the upper alimentary tract, colon, and skin.

#### HISTOLOGY

*Mouth:* The mouth lesions of the disease are readily accessible to observation during life and were naturally studied first. The development of the mouth lesions has been easiest to follow and observations made on them have been of material assistance in understanding the changes found in other tissues. They develop rapidly, change their character quickly, and thus the histology is more readily presented in narrative form. For the sake of brevity, reliance will be made on photomicrography rather than on detailed description.

The first changes appear in a narrow zone just beneath the epithelium and superficial to the subpapillary vascular plexus. Normally in the anterior portion of the dog's mouth the stroma just beneath the epithelium is compact, the vessels are small and inconspicuous, and there is but little difference in structure of the superficial stroma and the deeper fibromuscular tissue of the buccal wall. In the earliest recognizable lesions this zone becomes rarefied and unduly transparent as seen in microscopic preparations. The loss in density is due to the appearance of large intercellular spaces, widening of the vessels and loss of intercellular material — chiefly fine fibrillae. These changes are associated with redness of the mucous membranes seen during life.

The enlarged intercellular spaces were first looked upon as due to an edematous process, but this view has had to be revised because the process is not diffuse in the earliest lesions, and because the tissue spaces do not contain coagulable or stainable albuminous material. Edema fluid is readily coagulable with Zenker's solution and stainable with Giemsa's stain. Again, as the lesions spread and become more extensive the character of the intercellular material changes and it becomes coagulable with either Zenker's solution or formol. It is now thought that in the earliest phases of the lesions

the tissue spaces are filled with fluid too poor in albumen to be coagulable.

Particular effort has been made to understand the early degenerative changes in the fibrillae. It has not been possible to go beyond the observation that they become slender, shorter, and appear to fragment into converging rows of eosinophile dots. As these fibrillae are those which effect the attachment of the epithelium to the stroma, changes in the epithelium soon follow.

Alterations in the superficial vessels are as prominent as those in the fibrillar material of the stroma but they are secondary to them. The vessels become larger, their walls thin, and the endothelial cells long and slender. These changes give the appearance of a pathological vascularization of the stroma. They appear to result from degeneration of the supporting cells and fibrillae about the vessels and not to intrinsic changes in the walls. The impression is gained that the process is one of impaired vascular support rather than active engorgement.

The initial degenerative changes do not long remain in an uncomplicated state, for proliferation of perivascular cells soon becomes obvious and cells of the lymphocyte order appear in the intercellular spaces. So long as the epithelium remains intact migration of the polymorphonuclear leucocytes does not occur.

Degenerative changes in the covering epithelium appear clearly to be secondary to those in the stroma. The epithelial cells become pale, the intercellular spaces more readily visible, and the surface cells desquamate. The desquamation of the surface cells may reduce the epithelium to a single layer or complete denudation of the surface may result.

With loss in continuity of the epithelium the lesions assume a different appearance and become of a simple inflammatory type. A pseudomembrane forms on the surface made up of fibrin, cell debris and many forms of bacteria. Even when the surface epithelium is gone and the membrane has formed the lesions show but little tendency to suppurative reaction.

As shown in the summary statement in the appendix, several of the animals had had repeated attacks with recovery. In such cases active as well as partially healed lesions were found. Repair of the subepithelial stroma takes place by proliferation of connective tissue cells of larger size and with coarser fibrillae than those forming the

superficial stroma. It seems clear that the cells forming the stroma are more highly differentiated than most connective tissue cells and when destroyed are replaced by a simpler cell of the fibroblast type. The relations of the epithelium are not perfectly restored in repair but the stroma becomes simpler in structure with shorter and less highly developed vascular papillae. The more delicate vessels of the stroma are not replaced, but the coarser vessels of the subpapillary plexus come to lie closer to the epithelium.

*Tongue:* The dorsal surface of the dog's tongue is covered with coarse ridges of keratinized epithelium, which appear as curved barbs on section. These lie on a dense fibromuscular stroma. The inferior surface is covered with a thin epithelial coat and beneath this a stroma made up of delicate connective tissue. The difference in the structure of the stroma of the two surfaces appears to explain the well-nigh complete restriction of the lesions of the tongue to its inferior surface. The tongue lesions resemble those of the buccal mucosa and appear to pass through analogous phases.

*Pharynx:* The epithelium of the pharynx does not lie on the same type of stroma as in the anterior part of the mouth. A fairly broad zone of loose connective tissue containing lymphoid cells and small lymph follicles is interposed. For this reason rarefaction of the stroma is not so apparent, but even in early lesions the loss in density of stroma, fragmentation of fibrillae and the vascular changes previously described are visible. In more severe cases the epithelium becomes thin from degeneration and desquamation of cells and finally the stroma is denuded. Lesions of the pharynx appear to progress faster than those of the anterior part of the oral cavity. In sections including mucosa of both oral and nasal pharynx the lesions are confined to the hypopharynx.

*Epiglottis:* There is but little difference in structure of the upper and lower surfaces of the epiglottis. Lesions of the upper or pharyngeal surface are present in four cases, but they do not extend to the lower or laryngeal surface. The reason for this limitation in surfaces so contiguous is not clear.

*Esophagus:* Lesions of the esophagus are present in three cases and are similar to those of the buccal mucosa.

*Respiratory Tract:* In three dogs killed late in the series there are changes in the small bronchial radicles. The epithelium has lost its columnar character, the cells are enlarged and irregular in shape

and they fuse to form large giant cells about a central mass of mucus. The walls of these radicles are thin, the muscle coats are hyalinized and no lymphoid stroma remains between the epithelium and the supporting tissue. No other changes have been observed in the respiratory tract.

*Stomach and Small Intestine:* Comparison of sections from early and late cases show a progressive loss of density in the stroma of the villi. This appears to be due to the decrease in the lymph and reticulum cells. A colloid substance has diffused among the cells and fibrillae. This material while staining somewhat similar to mucus does not appear to be a product of the epithelium. Whether it is derived from the connective tissue of the stroma or is some plasma derivative is undetermined. Its distribution and appearance are similar to that of the intercellular material found in the subepithelial stroma of the mouth.

*Large Intestine:* In the later dogs of the series there are definite changes in the colon. The colic villi are distorted. Some are slender and appear compressed while those adjacent may be broad and bulbous. The lymphoid stroma is uniformly decreased. Between the villi are numerous mucus-filled cysts, formed by distention of crypts. In the broader villi the vessels are dilated and form small varices beneath the epithelium. There is great irregularity in the shape of the epithelial cells; some are oversized and triangular in shape, others are flat and elongated, while those lining the small cysts in the mucous membrane are stretched and distorted by distension.

*Skin:* Changes analogous to those observed in the buccal mucous membrane are present in sections of the skin from the scrotum. In three cases the corium shows the same rarefactive changes seen in the buccal lesions. A late case shows repair of the superficial collagen by proliferating fibroblasts. As in the mouth the process appears to begin in the fibrillar material supporting the epithelium.

*Nervous System:* No definite changes have been observed in the central nervous system. The number and arrangement of the tigroid granules in the large ganglion cells of the brain and cord are apparently subject to some variation normally. No striking change in the tigroid substance or in the nerve cells has been found in the animals of this series.

*Other Organs:* Nothing of significance has been found in the other tissues or organs of the animals examined.

## CONTROLS

As shown in the appendix, Dog 100 was killed at the outset of the experiment as a normal control. Additional normal material was obtained from animals provided by the Department of Health at Yonkers, New York. Histological appearances comparable to those described have not been seen in the normal animals.

Dog 76, suffering from a polyneuritic condition induced by a deficient "synthetic" diet was extremely emaciated and partially paralyzed. Mucous membrane and cutaneous lesions were not observed clinically and histological preparations show no special changes in these tissues. Sections of the spinal cord stained by Weigert's method show a degenerative process in the pyramidal tracts.

## SUMMARY

The lesions of experimental black tongue are located in the mucous membranes of the mouth, pharynx, esophagus, intestine, and skin of the scrotum. They originate in a degenerative process affecting the superficial connective tissue of the mucous and dermal membranes of these respective surfaces. Changes in the supporting tissues of these mucous membranes are followed by secondary ones in the epithelium. The lesions tend to terminate in an extensive necrotic and diphtheritic inflammation of the upper alimentary tract.

## DISCUSSION

On account of the similarity in the appearance clinically of the mouth and skin lesions of experimental black tongue to those of pellagra in man (Goldberger and Wheeler) it may be of value to compare the microscopic features of the lesions of the two diseases.

The pellagra material available for comparison was collected by the present writer in Panama in 1923 and described in 1924.<sup>3</sup> It has been completely restudied in connection with the present investigation, but no important additions can be made to the observations originally reported. The special value of this human material lies in the fact that the cases studied were typical and uncomplicated cases of pellagra. The patients were admitted to the hospital suffering with pellagra and four died while still in good stages of nutrition. The salient features of the observations reported may be briefly summarized as follows:

*Skin:* The early skin lesions in the stage of redness and swelling, usually referred to as the erythematous stage, owe their peculiar characters not to acute inflammation (in the ordinary sense of the term) but to a rarefactive process in the superficial corium and to widening of the superficial vascular channels. The earliest tissue change is a disintegrative process affecting the fibrillar structures which constitute the binding substance between the corium and epidermis. This process may be so complete as to bring about separation of the epidermis over extensive areas. In milder lesions the disintegrative process and separation of the epidermis may be confined to microscopic areas. The thickening of the skin and the scaling in the later cases is due to abortive or imperfect repair of the corium. Healed lesions usually show atrophy of the skin with marked thinning of the epidermis and often with telangiectases in the cicatrized corium. The skin lesions show no evidence of being infectious in origin and often remain aseptic throughout their course.

*Mouth, Pharynx and Esophagus:* The remarkable feature of the lesions of the upper alimentary tract is their similarity to the skin lesions. They have their basis in degenerative changes in analogous fibrillar and cellular structures. The striking red color of the mouth and pharynx is perfectly analogous to the erythema of the skin. The fact that the mouth lesions do not remain aseptic is responsible for the apparent differences in the lesions.

*Intestine:* Lesions of the intestine, most marked in the colon, while not bearing superficially a resemblance to those of the mouth and skin, are, on analysis, quite similar. The supporting elements of the mucous membrane are the structures primarily affected. Secondary, degenerative changes in the epithelium result in denudation of the stroma with the formation of a diphtheritic membrane. Small diphtheritic lesions tend to spread and terminate as larger and deeper ones. In later cases the colon may show marked atrophic changes with reversion of the mucous membrane to a simpler type, often with small mucous cysts in the mucosa. Telangiectases like those in the corium are found in the colonic mucosa in later cases.

*Nervous System:* No distinctive changes are found in the central nervous system in the earlier cases. In the later ones in which there had been repeated attacks and in which the patients died in an emaciated stage there are hyaline changes in the small vessels and chromatolytic changes in the ganglion cells of the brain and cord.

In general the alterations in the central nervous system are in inverse proportion to the acuteness and extent of the lesions of the skin and alimentary tract.

It appears that entirely too much significance has been attached in the literature to the rather indefinite changes described in the nervous system, especially since definite information is lacking as to the permanence of the alterations in the chromatic substance of the nerve cell to which the term chromatolysis has been applied. The majority of the studies of the central nervous system in pellagra have been based on material collected in insane asylums.

#### COMPARISON OF THE LESIONS OF PELLAGRA WITH THOSE OF EXPERIMENTAL BLACK TONGUE

The lesions of the skin, mouth, pharynx, esophagus and colon in pellagra and in experimental black tongue in dogs show very similar gross appearances. Histologically the lesions of both appear to have their inception in a degenerative process in an analogous tissue element. The processes of repair in both result in fibrotic replacement and in pathological vascularization of the superficial stroma of the mucous membrane of the upper alimentary tract and of the corium. The lesions of both are primarily degenerative in character and have the same tendency to secondary infection.

The lesions in the experimental condition in the dog render the lesions of pellagra more understandable because the former can be obtained earlier and are less complicated by infection than in man.

The distinctive lesions of pellagra and those of black tongue of dogs appear to have their origin in a failure on the part of the organism to maintain the specialized supporting tissues of epithelium in various situations.

#### APPENDIX

*Summary of significant details in the history of each of the dogs from which tissues were secured. Furnished by Surgeon, Joseph Goldberger, U. S.*

##### *Public Health Service*

*Dog 13: Male. Between April 7, 1923, when acquired, and April 28, 1927, when killed, had in all eight attacks of experimental black tongue. The last attack began January 25, 1927, or 48 days after beginning the experimental diet. This was a relapsing attack accompanied by self-imposed semistarvation. Weighed 9.4 kilograms when last attack began, and 6 kilograms 2 days before he was killed. Had an intercurrent infective ulcerative stomatitis between March 15 and April 17, 1927.*

*Dog 15:* Male. Between April 14, 1923, when acquired, and July 12, 1927, when killed, this animal had in all four attacks of experimental black tongue. The last attack began June 28, 1927, that is 117 days after beginning the experimental diet. This was a relapsing attack of moderate severity. Killed with illuminating gas, July 12, 1927.

*Dog 42:* Male. Between June 26, 1923, when whelped in the laboratory, and October 16, 1926, when killed, had in all four attacks of experimental black tongue. The last attack began September 28, 1926, or 56 days after beginning the experimental diet. In dying condition was killed with illuminating gas. Weighed 12.8 kilograms when last attack began, and 11.5 kilograms 4 days before he was killed.

*Dog 62:* Male. Whelped in the laboratory November 4, 1923. Up to June 12, 1926, had had no recognizable black tongue. In good condition June 12, 1926, when experimental diet was begun. Killed with illuminating gas June 26, 1926. No signs of black tongue during the observation period of 14 days.

*Dog 66:* Male. Between November 25, 1923, when whelped in the laboratory, and January 18, 1927, when killed, had in all four attacks of black tongue. The last, a chronic, mild, relapsing attack with a flaccid paraplegia accompanied by semistarvation began December 3, 1926, about 10 months after beginning an experimental diet. Weighed 9.8 kilograms on November 30, 1926, 2 days before the beginning of the last attack, and 7.5 kilograms on day when killed with illuminating gas.

*Dog 69:* Male. Whelped in the laboratory November 25, 1923. Developed the first and only attack of experimental black tongue July 28, 1926, or 46 days after beginning the experimental diet. Killed with illuminating gas on August 7, 1926, on the 10th day of a mild developing black tongue.

*Dog 76:* Male. Between June 9, 1924, when acquired, and June 29, 1927, when killed, had in all one relapsing attack of experimental black tongue, which began September 4, 1926, from which he fully recovered. On December 8, 1926, began an antineuritic deficient "synthetic" diet. On January 27, 1927, that is, at the end of 50 days, this animal developed signs of "polyneuritis." Two days later, when in dying condition, was killed with illuminating gas.

*Dog 82:* Bitch. Between October 13, 1924, when acquired, and January 30, 1927, when this animal died, she had had in all three attacks of experimental black tongue. The last attack began January 22, 1927, or 45 days after beginning the experimental diet; a rapidly progressive attack. The animal died sometime between 9 P.M., January 29, and 1 A.M., January 30, 1927.

*Dog 100:* Male. Whelped in the laboratory December 9, 1925. Reared on a stock diet. Normal animal. Killed with illuminating gas, June 26, 1926.

*Dog 101:* Male. Whelped in the laboratory December 9, 1925; litter mate of Dog 100. Reared on stock diet. In good condition June 12, 1926, when the experimental diet was begun. First sign of a mild relapsing progressive attack appeared July 10, 1926. Killed with illuminating gas August 7, 1926.

*Dog 102:* Male. Litter mate of Dog 100. Reared on a stock diet. In good condition June 12, 1926, when the experimental diet was begun. First sign of

a moderately severe, rapidly progressive attack of experimental black tongue appeared July 10, 1926. Killed with illuminating gas July 20, 1926.

*Dog 103:* Male. Litter mate of Dog 100. Reared on a stock diet. In good condition June 12, 1926, when the experimental diet was begun. First sign of relapsing, moderately severe attack of experimental black tongue appeared July 10, 1926. Killed with illuminating gas August 7, 1926.

*Dog 104:* Bitch. Litter mate of Dog 100. Reared on a stock diet. In good condition June 12, 1926, when the experimental diet was begun. Mild beginning experimental black tongue, July 6, 1926. Killed with illuminating gas July 10, 1926.

*Dog 105:* Bitch. Litter mate of Dog 100. Reared on a stock diet. In good condition on June 12, 1926, when the experimental diet was begun. A mild relapsing experimental black tongue began July 15, 1926. Killed with illuminating gas August 7, 1926.

*Dog 106:* Bitch. Litter mate of Dog 100. Reared on a stock diet. In good condition June 12, 1926, when the experimental diet was begun. Killed with illuminating gas June 26, 1926. No signs of black tongue during the observation period of 14 days.

*Dog 109:* Bitch. Acquired October 29, 1926. A fatal attack of experimental black tongue began December 30, 1926, 22 days after beginning the experimental diet. The attack was a relapsing one accompanied by prolonged self-imposed semistarvation. Weighed 7.9 kilogram on December 28, 1926, and 3.4 kilograms on April 19, 1927. Died April 20, 1927.

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2. Goldberger, J., and Wheeler, G. A. *Pub. Health Rep., U. S. Pub. Health Service, Washington, D. C., 1928*, xliii, 172.
3. Denton, J. *Am. J. Trop. Med.*, 1925, v, 173.

## DESCRIPTION OF PLATES

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### PLATE 80

FIG. 1. Dog 101. Buccal mucosa, margin of an early lesion. Rarefaction of the subepithelial stroma and degenerative changes in the epithelium.

FIG. 2. Dog 104. Buccal mucosa, early lesion. Fragmentation of the fibrillar material contiguous to the basal layer. Note the very limited changes in the epithelium.



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2

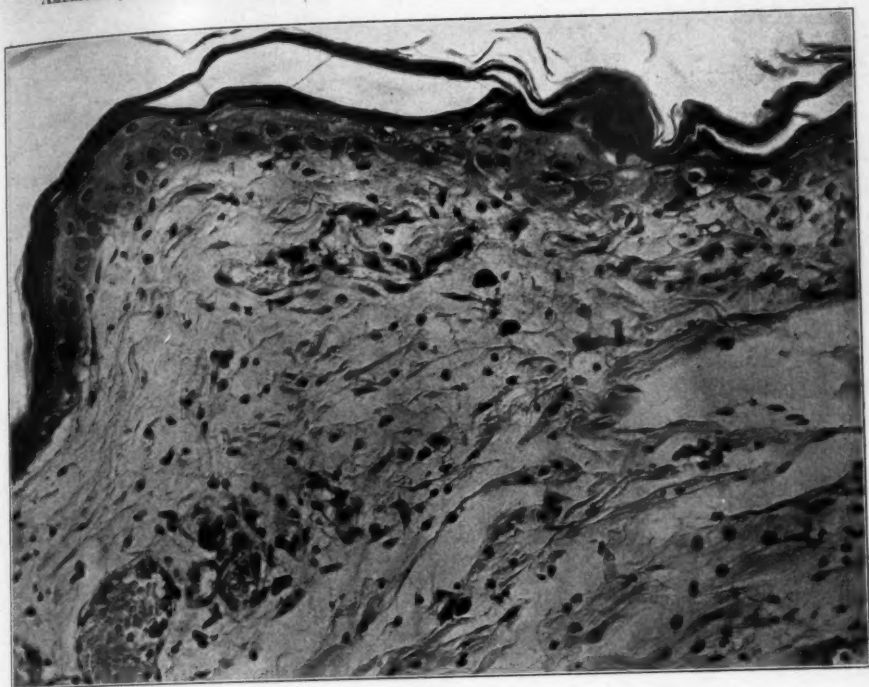
Denton

Experimental Black Tongue of Dogs

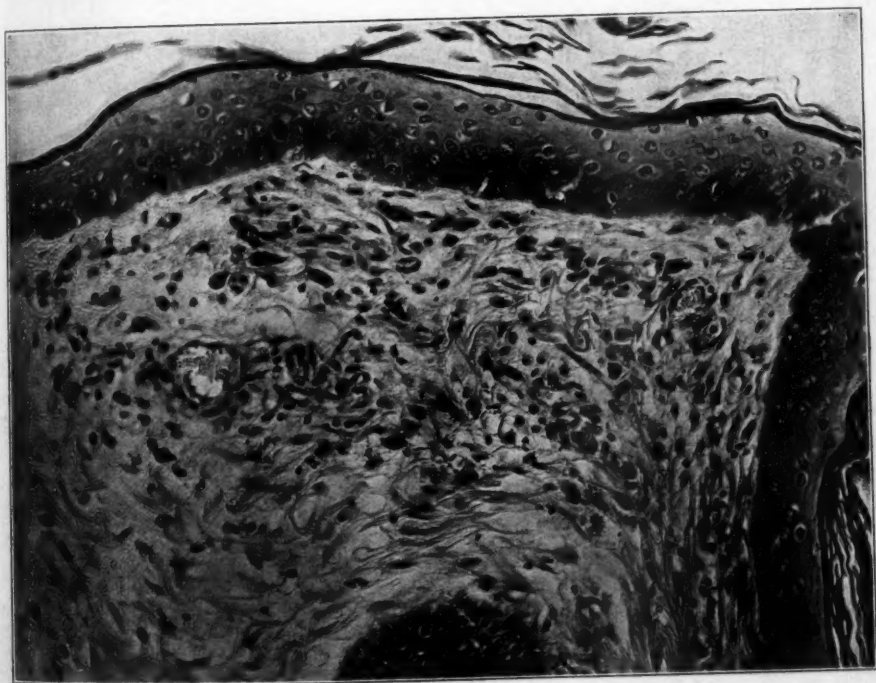
PLATE 81

FIG. 3. Dog 103. Skin, scrotum. Changes in the corium similar to those in the subepithelial stroma shown in Fig. 2.

FIG. 4. Dog 15. Skin, scrotum. Rarefaction of the corium and degeneration of the superficial fibrillae.



3



4

Denton

Experimental Black Tongue of Dogs

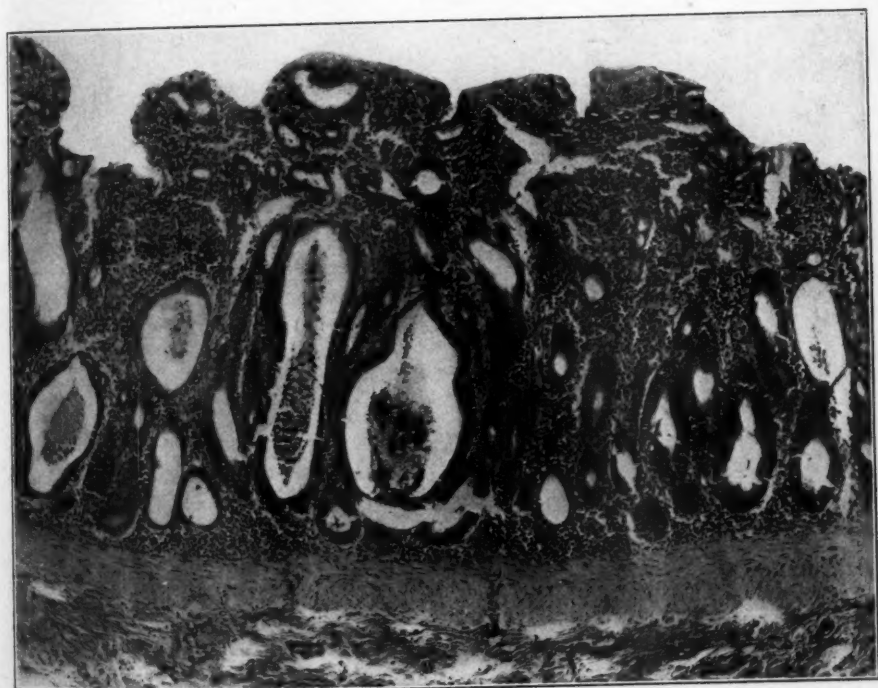
PLATE 82

FIG. 5. Human skin. Skin from pellagra in man. Note the similarity in the changes in the corium to those in the buccal mucosa and in the skin of the scrotum in the dog.

FIG. 6. Dog 109. Colon, late lesion. Aplasia of the villous stroma, regressive changes in the intestinal epithelium with formation of mucous cysts in the dilated glands.



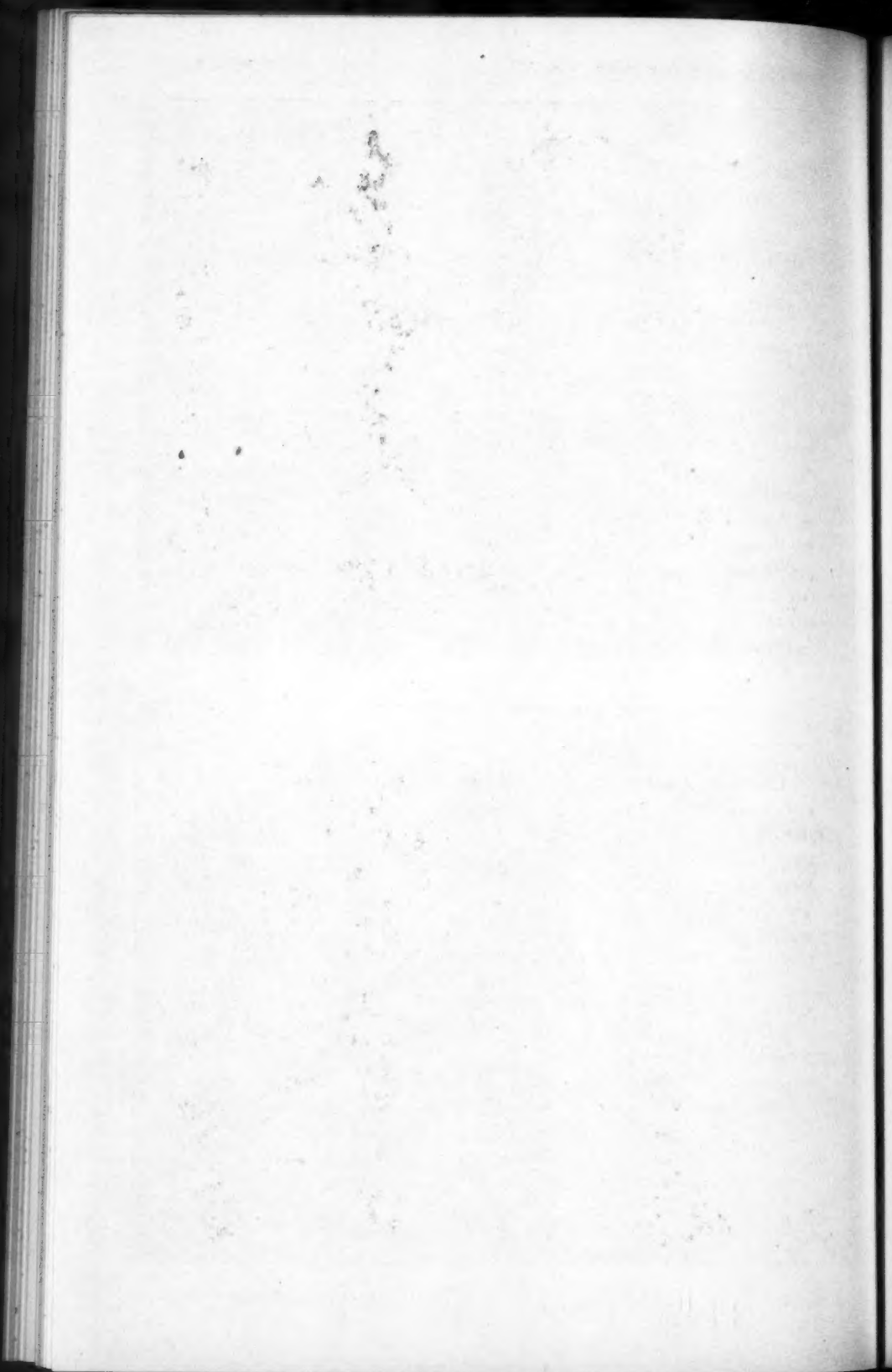
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Denton

Experimental Black Tongue of Dogs



## OBSERVATIONS ON BLOOD INCUBATED UNDER ABNORMAL CONDITIONS \*

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In two previous papers<sup>1,2</sup> we have reported our observations on incubated leukemic and normal blood. In the experiments described below, we studied the effect of various substances and different cultural conditions on the leucocytes of incubated normal human and rabbit blood. In our preceding papers, we described the development of two types of large cells in both leukemic and normal blood. We termed these two types of cells "X" and "Y." The "X" cell was thought to be derived from a pluripotential, embryonic blood cell, while the "Y" cell developed from the monocyte. This brief mention of these cells is made here as the terms are used in certain experiments in this paper. We have described in detail in our other work the terms "monocyte," rosette," etc.

### METHODS

Blood, removed from the median basilic veins of humans or from the hearts of rabbits, was placed in small tubes and allowed to clot, or it was added to tubes containing sodium citrate or heparin and then treated in different ways described later. In every experiment the tubes were sealed with paraffin before incubation. Specimens for examination were removed with a platinum loop at various times. These specimens were studied by the supravital technic of Sabin *et al.*, and in air-dried smears by Wright's stain and Sato and Sekiya's peroxidase reaction.

### OBSERVATIONS

Each single experiment in the various series will not be gone into in detail but a composite description of the results in each group will be given. The heading of each set of observations indicates the substance or condition the effect of which was being studied.

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## ICEBOX TEMPERATURE

In the experiments previously reported the bloods were incubated at  $37.5^{\circ}\text{C}$ . The question arose as to the significance of the large cells that appeared. Were they merely degenerating forms or were they the result of physiological activity? If they were the former, it seemed probable that they would appear under conditions where the leucocytes could be kept alive for a period of time with their physiological activities diminished or suppressed. Icebox temperature seemed to offer such conditions, for others have shown that tissues can be grown after being kept in the icebox for at least eight days. Accordingly experiments were set up with clotted rabbit blood, one set of tubes being kept at  $10^{\circ}\text{C}$ , another set as controls at  $37.5^{\circ}\text{C}$ . The results of the tubes kept at  $10^{\circ}\text{C}$  were as follows: Leucocytes of the different types were found alive at the end of three weeks but not four weeks. From the sixth day on an increasing number of dead cells of various kinds were found. The polymorphonuclear neutrophils as time went on contained globules staining with neutral red, situated at the periphery of the cells. The lymphocytes remained normal in size but there was some increase in the size of their neutral red granules. The monocytes likewise showed no change in size but their rosettes became larger due to the increased size of the neutral red granules. No refractile granules, which were so commonly seen in these cells at incubator temperature, were noted. No large cells of any type or transition forms were ever found. In one experiment, a tube that had been incubated eleven days at  $37.5^{\circ}\text{C}$  and that contained a considerable number of large cells was placed at  $10^{\circ}\text{C}$  and observed for six days. The cells remained alive and showed no change in their morphology or size. No tendency to revert to the forms from which they were derived was noted.

The results of these experiments would seem to confirm the view that the large cells are the result of physiological activity and are not merely degenerative forms.

## SODIUM CITRATE AND HEPARIN

One of the difficulties we encountered in working with clotted blood was the scarcity of leucocytes in our preparations during the first few days of incubation. As time went on, leucocytes appeared in larger numbers. Apparently the cells were caught in the clot at

the beginning and gradually migrated out into the serum. To obviate this difficulty we decided to use an anticoagulant, sodium citrate. This was employed at a concentration of 1 per cent in Locke's solution and an equal volume of blood was added. For the first few hours after the addition of blood to this solution, the leucocytes were rounded up and non-motile. After twenty-four hours' incubation they had regained their normal appearance and motility. However, after forty-eight hours they were all dead, showing a peculiar ballooning of their cytoplasm. It seemed as if it took the leucocytes some time to adjust themselves to this solution, but even after they had done so they could live but two days. If the citrated blood was centrifuged shortly after citrating and about two drops removed from the upper layer of the sediment and added to 1 cc. of serum, the cells survived for long periods with the development of many large cells. However, clotting occurred under these conditions and made observation difficult.

After our experience with sodium citrate, we decided to try heparin, which has been so useful in physiology and for tissue cultures (Craciun<sup>3</sup>). Heparin was dissolved in Locke's solution and sterilized at fifteen pounds pressure for fifteen minutes in the autoclave. At first, we used final dilutions of 1:15,000 as recommended by Craciun for heparinized plasma but we found that such a concentration did not prevent the blood from clotting. Eventually we found that a final dilution of 1:600 or 1:800 was satisfactory for human blood. Rabbit blood always clotted at such dilutions. The fact that whole blood will clot at concentrations of heparin that prevent clotting of plasma alone, suggests that the cells of the blood must yield substances causing clotting similar to those of tissue cells. The presence of heparin did not affect either the periods of survival of the various types of leucocytes or the development of the large cells.

#### OLD CULTURE FLUIDS

Each type of leucocyte had a rather definite period of survival in our experiments on incubated blood. Also large cells made their appearance at about the same time in each experiment. The question naturally arose: Was the length of life of the leucocytes and the development of the large cells dependent on the accumulation during incubation of waste products in the medium? If this were the case,

then renewal of the fluid medium should prolong the life of the cells, and conversely, the use of fluids in which cells had been incubated should shorten the life of the cells. Also such changed conditions should affect the development of the large cells. In several instances, therefore, we removed the fluid after centrifuging the tubes slowly, and added fresh fluid of the same composition. The results were inconclusive; no definite effect could be noted. We then tried the other method of attack. Tubes that had been incubated respectively eight days and two weeks were centrifuged. The supernatant fluids were removed and added to equal volumes of fresh, heparinized human blood. The blood in all sets of tubes was taken from the same individual. These supernatant fluids were dark brown-red owing to hemolysis. After twenty-four hours incubation, the monocytes and also some of the polymorphonuclear neutrophils contained many granules of what appeared to be hemoglobin. (Phagocytized granules of similar appearance were often seen in cells in the usual incubated blood tubes after a weeks incubation when considerable hemolysis had taken place.) The monocytes were either oxidase-positive or negative, whereas in the control tubes all the monocytes were oxidase-positive. The general condition of the cells was about the same as in the control tubes. Incubation was continued for three weeks. The different types of leucocytes survived the same periods of time as in the controls. These different periods were the same as those we have found in our other experiments. "X" cells appeared earlier and occurred throughout in greater numbers than in the controls. There were also more "Y" cells than in the controls.

From the result of these experiments, it would appear that the death of the cells under the conditions of our cultures was dependent on their natural length of life, not on the accumulation of waste products, and the presence of old culture fluids favored the development of "X" and "Y" cells. In a way our results agree with those of Awrorow and Timofejewskij.<sup>4</sup> These workers found in culturing leukemic blood that subcultures to fresh medium did not prolong the life of the cells.

#### LITHIUM CARMINE AND TRYPAN BLUE

These experiments were carried out with clotted rabbit blood. A few drops of 5 per cent lithium carmine or 1 per cent trypan blue

were added to the tubes after the blood had clotted. In one experiment, a rabbit was bled five minutes after receiving intravenously 5 cc. of unfiltered lithium carmine solution. The serum of this specimen was highly colored with the dye but there was no carmine in any of the leucocytes. Tubes in each series, with and without the dyes, were incubated at  $37.5^{\circ}\text{C}$  and specimens were studied on both unstained slides and slides with neutral red.

The dyes had no apparent effect on the duration of survival or development of the cells. Large cells appeared in approximately the same number and of the same morphology as in the controls. In one experiment, there was a suggestion that the cells in the tubes containing the dyes were more phagocytic but this may have been chance and it was noted in only this one experiment. Neither carmine nor trypan blue was taken up as such in any appreciable amount by any type of cell. When examined on unstained slides, the large cells sometimes showed some fine granules of dye in their rosette areas. The dyes stained dead cells, and such stained dead cells and debris were frequently found in the large cells. Whether this phagocytized material was stained before or after phagocytosis, it is impossible to say. Granules or masses of dye, except those mentioned above, were never found in the cells. In an experiment carried out at  $10^{\circ}\text{C}$  with carmine, no dye was found in the cells nor had it changed the cells in any way.

#### HIGGINS' AND WEBER'S INDIA INKS

Muller<sup>5</sup> has recently described the effect of repeated injections of these two inks. There was a striking difference in the results with the two inks which apparently was dependent on the protective colloid present, not on the carbon. We felt it would be of interest to compare the two inks in their action on the development and survival of the large cells of incubated blood. Clotted rabbit blood, 2 cc. to a tube, was used and one loopful of ink was added to a tube. Controls without ink were always run. The results were striking. Large cells developed normally in the presence of Higgins' ink whereas no such cells were found in the tubes containing Weber's ink. The differences in the effects of these two substances was probably to be ascribed to the particular protective colloid in Weber's ink. Higgins' ink occurred in the large cells both as very

fine granules in the rosette area and in larger masses at the periphery of the rosette. Such ink rosettes on an unstained slide simulated closely in size, number and arrangement of granules those stained by neutral red. Observed on a neutral red slide, the ink in the rosette area was slowly replaced by the neutral red until finally no trace of the ink could be seen. This bringing out of rosettes by Higgins' ink was observed in another kind of experiment. In this, a drop of diluted ink was added on a slide to a drop of incubated blood that contained many large cells. After four hours' incubation, the rosettes of the large cells were delineated clearly by the ink particles, and were identical in appearance with rosettes stained by neutral red. It would seem, therefore, that certain colloidal solutions such as Higgins' ink, and to a less extent carmine and trypan blue, occur as fine particles in the rosette areas, possibly deposited on the same granules of the cells that neutral red stains.

#### CARBON

We hoped by allowing cells to phagocyte carbon and then doing an oxidase reaction to be able to distinguish two types of phagocytic mononuclear leucocytes — one oxidase-positive, the other oxidase-negative. Carbon, in the form of lampblack, was added to heparinized human blood. The tubes were incubated at 37.5° C. After two hours incubation, some of the polymorphonuclear neutrophils and monocytes contained granules of carbon. After four hours, the polymorphonuclears had phagocytosed a little more carbon. The monocytes contained varying amounts of carbon. Where it was small in amount and in fine granules, it occurred at the periphery of the rosette. When the amount was larger and the granules coarser, no rosette could be seen. In some of these cells, a few fine neutral red granules could be made out above or below the nucleus, but in others no neutral red granules could be found in any part of the cell. When examined with the oxidase reaction, the monocytes with a few carbon particles were found to be all oxidase-positive. Other monocytes that contained more carbon had fewer oxidase granules and some that contained the most were apparently oxidase-negative. In other words, the number of oxidase granules present was in inverse proportion to the amount of carbon in the cells. The oxidase reaction paralleled closely the neutral red picture, *i.e.*, the more the phagocytosed carbon particles, the fewer the stainable granules.

Stained by Wright's method, practically all the monocytes contained carbon. There was a marked tendency for these cells to occur in clumps around large masses of carbon. At twenty-four hours, in the supravital preparations, the monocytes were grouped around carbon masses. Their rosettes were obscured or absent. The phagocytosed carbon tended to be at the periphery of the cell if there was not too much present. Some of the finest granules occurred in the rosette areas. In preparations stained with both neutral red and Janus green, no mitochondria could be seen in the monocytes although numerous in the lymphocytes. In the fixed smears, all monocytes contained carbon and all were oxidase-negative. At three days, most of the monocytes were dead while at four days they were all dead. The other types of leucocytes were living and normal and survived their usual periods. A few "X" cells developed, but no "Y" cells.

We have described the above in such detail because it brings out two points of importance. The first is the apparent or real loss of the monocytes' neutral red rosettes, so characteristic of this cell, following the ingestion of carbon. The second is the change in the oxidase reaction from positive to negative in these phagocytic monocytes.

In short, after taking up carbon, the monocytes lost the two characteristics by which they are recognized — rosette formation and oxidase reaction. The importance of the possibility of such changes is obvious, for one could easily be misled in attempting to identify such cells without having followed them from the beginning. Their negative oxidase reaction, lack of rosette and marked phagocytosis would place them in Sabin's clasmatoocyte or endothelial cell group, a type of cell held by her school to be quite distinct from the monocyte group.

#### TUBERCLE BACILLI

Some living avian tubercle bacilli were added to clotted rabbit blood tubes. Large cells had developed at five days and they contained bacilli. The rosettes of the large cells tended to be somewhat broken up and irregular. The bacilli occurred between the neutral red granules at the periphery of the rosette and were often arranged radially with respect to the centrosphere. These cells remained alive but half as long as those in the controls. Living tubercle bacilli,

human strain H 37, were added to heparinized human blood. After five hours incubation, the monocytes were found grouped about clumps of bacilli. The granules of the rosettes of these cells were larger than normal and somewhat irregular in size. The other type of leucocytes appeared normal. At twenty-four hours, a stain for tubercle bacilli followed by Wright's stain showed the monocytes containing masses of tubercle bacilli and a few of the polymorphonuclear neutrophils with single bacilli or small clumps. In both the supravital and Wright's stain, the monocytes looked in poor condition while the other leucocytes appeared normal. The monocytes were oxidase-positive. All the monocytes were dead at three days. The polymorphonuclear neutrophils and eosinophils lived seven days. The lymphocytes could be followed twelve days and they remained unchanged in appearance during this time. The bacilli grew freely in the fluid. No large cells developed.

#### FOREIGN ERYTHROCYTES

Phagocytosis of red cells by "X" and "Y" cells was a common phenomenon in incubated blood. The red cells were sometimes taken up unchanged. At other times, phagocytic cells were found containing eight to twelve completely hemolyzed erythrocytes. Whether hemolysis took place before or after phagocytosis is difficult to say, but probably the former was usually true, for at such times practically all the red cells in the tubes were completely hemolyzed. Also, it was rare to find cells containing so many unhemolyzed red cells. In some experiments designed to study the production of antibodies by the blood leucocytes, sheep blood was added to incubated blood tubes at various times. Phagocytosis of these foreign red corpuscles was often active. Such phagocytosis was also used by us as a test for the physiologic activity of the large cells. Such a test had significance as it was possible that the large cells were inactive, degenerating forms. In one such instance, a small amount of sheep blood was added to tubes of rabbit blood that had been incubated eleven days and contained many large cells. Twenty-four hours later, the tubes were examined and showed marked phagocytosis of the sheep red corpuscles by the large cells. Some contained as many as thirty red cells. The method of phagocytosis was observed in the supravital preparations. The peripheral portion including the pseudopods

of the large cells consisted of clear, non-granular cytoplasm. A red cell would be taken into this clear portion and moved up to the edge of the granular area. At this point, the red corpuscle was either taken into the granular region and placed at the periphery of the rosette or it was violently expelled out of the cell into the surrounding fluid. Cells were seen to take up and reject several red cells in succession before one was taken into the granular area. Some large cells were observed to admit to the granular area as many as three red cells at the same time. It seemed as if the red cells were taken into the clear cytoplasm tentatively. Then some factor decided whether they should be taken up permanently or whether they should be expelled immediately. Erythrocytes never remained any length of time in the clear zone.

#### SUMMARY

The effect of the addition of various substances on the physiologic activities and development of large cells in incubated normal rabbit and human blood is described. These substances included carmine, trypan blue, Weber's and Higgins' inks, carbon, tubercle bacilli and old culture fluids. The periods of survival and changes of the different types of leucocytes at 10° C are reported. The use of citrated and heparinized plasma and their effect on leucocytes are discussed.

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TWO OSTEOLASTOMAS NOT CONNECTED WITH BONE, HISTOLOGICALLY IDENTICAL WITH OSTEOLGENIC SARCOMA, AND CLINICALLY BENIGN \*

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In the eight years which have elapsed since the establishment of the Bone Sarcoma Registry by Codman the interest of both pathologists and clinicians in tumors of bone has been steadily increasing. Due to the pioneer work of Codman and the recent review of Kolodny<sup>1</sup> a great mass of well organized and classified material is now available.

During the past two years two patients have been under treatment at the Boston City Hospital who have had tumors in the thigh not attached to bone. Microscopic examination has shown the tumors fulfil every criterion used for the establishment of a diagnosis of osteogenic sarcoma. They have been completely removed surgically and now at the end of two years for one, and six months for the other, there has been no evidence of recurrence.

The diagnosis of tumors of the type of osteogenic sarcoma is often based on the histologic findings. When such a diagnosis is made the prognosis for the patient is so bad that we have felt that these two cases deserve to be put on record.

**CASE I. Clinical History:** The patient, S. L., female, unmarried, a department-store worker, 21 years of age, entered the hospital August 17, 1926, under the care of Dr. Robert Cochrane. She complained of a lump on the right thigh of five weeks duration. The family history, past history and habits were irrelevant.

The present illness began about five weeks before her admission to the hospital. The patient was in the habit of carrying a pocket filled with coins. As she walked, this heavy weight repeatedly struck her right thigh. Her attention was first called to that region of her body by the development of a slight pain felt only when she moved her leg. On examination she felt a firm, slightly tender mass about the size of an English walnut in the location which was struck by the coins. She thought that there had been a definite increase in the size of the tumor in the time which elapsed between her first observation of it and her

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entrance to the hospital. Except for the loss of about ten pounds in weight during the previous six months she had noticed nothing wrong and had felt perfectly well.

Physical examination showed a fairly well developed and well nourished girl in no distress. The head, chest, neck, arms and abdomen were negative. No lymph nodes were palpable. Examination of the bones revealed no abnormality. On the inner, upper aspect of the right thigh over the adductor group of muscles there was a firm, oval mass about 5 cm. in the greatest diameter. The tumor was very indurated, discrete and firmly attached to the underlying structures. No acute tenderness or fluctuation was observed. Slight pain could be elicited by deep palpation. The skin over the mass was not abnormal.

X-ray studies of all bones were negative.

The preoperative diagnosis was "bone tumor."

Operation was performed under gas-oxygen anesthesia. The skin and subcutaneous tissues were retracted and a tumor mass about 5 cm. long was displayed. It was oblong in shape, very firm, an even gray in color, and firmly attached to the adductor magnus muscle. The mass was dissected free and shelled out very easily. Little bleeding was encountered.

The postoperative course was uneventful and the patient was discharged on the ninth day.

Complete physical examination and X-ray studies of the bones, done two years after operation, show no evidence of recurrence. The patient has been perfectly well and active.

#### PATHOLOGIC REPORT

*Gross Description:* The specimen consists of an irregularly oval mass of pink tissue with a rough surface. Consistence is very uniform and hard. Cut section is pink, mottled with gray translucent areas. Considerable calcification is present.

*Microscopic Examination:* The background of the tumor consists of narrow, elongated, spindle-shaped cells, mostly of a fairly adult type with an oval nucleus and a small amount of acidophilic cytoplasm. The nuclear chromatin is small in amount and rather scattered. In the preparations stained with phosphotungstic acid-hematoxylin the fibroglia fibrils are very clearly seen. The intercellular substance is composed of delicate collagen fibers grouped in parallel rows to form wavy strands. There is a tendency to form bundles which run at right angles to each other. There are a few fat cells and occasional groups of lymphocytes present. In certain areas every stage in the transition from connective tissue to osteoid tissue and from osteoid tissue to bone may be seen. On the collagen between the connective tissue cells, strands and small masses of smooth brown-staining material are laid down. These strands anastomose and the size increases. The fibroblasts in these regions

have become enlarged and are quite variable in appearance. They have lost their fibrils and have become oval to fusiform in shape. The cytoplasm is greater in amount and often is basophilic. The nuclei are larger, more irregular in size, and contain a large amount of chromatin. Mitotic figures are quite plentiful although no multiple mitoses are seen in this tumor. As the intercellular substance increases in amount single and paired cells are isolated and appear in small lacunae. As the bone more nearly approaches the adult type the intercellular substance takes a brighter pink with eosin and the cells decrease in size to appear like typical bone cells. Around the bone spicules rows of osteoblasts are arranged laying down more hyaline substance. A fair number of small, well formed vessels are present. The appearance of the bone is variable. It is for the most part unorganized, atypical, and of neoplastic type, although a small amount of normal-appearing adult bone is seen. Foreign body giant cells are present in small numbers. No muscle fibers are seen.

**CASE II. Clinical History:** The patient, L. K., a married business man, 37 years of age, entered the hospital December 2, 1927, complaining of a swelling in the left groin, of one years duration. The family history, social history, past history and habits were irrelevant to the present illness.

The condition began twenty-eight months before admission to the hospital when he struck his left groin on the sharp corner of a piece of furniture. A tender, bruised area resulted which subsided after several weeks without leaving signs or symptoms. About fifteen months before admission he first noticed a firm lump about 1 cm. in diameter on the inner side of the upper left thigh at about the same location as the previous bruise. The mass gradually grew larger but caused no discomfort until three weeks before entry when he noticed that pressure on it caused slight pain. He felt perfectly well and had no discomfort.

Physical examination showed a well developed and well nourished man in no distress. The head, neck, arms, chest and abdomen were negative. Both testicles were in the scrotum and were negative to palpation. The only lymph node palpable was one about 4 mm. in diameter in the left inguinal region, which was not adherent or tender. Examination of all bones was negative. On the inner aspect of the upper left thigh there was an extremely hard, smooth, rounded mass measuring roughly 2 by 2 by 6 cm. It was quite superficial and not adherent to skin or underlying tissues. The skin over the mass was normal in appearance.

Examination of the blood and urine showed nothing abnormal. Blood Kahn and Wassermann reactions were negative.

X-ray films of the chest and all the bones of the skeleton showed no abnormality. X-ray examination of the tumor showed an area of increased density corresponding in size to that of the tumor mass itself.

The provisional diagnosis was fibroma of the fascia.

Operation was performed by Dr. Donald Munro, December 4, 1927, under local anesthesia. The mass lay just under the skin in no way connected with underlying muscle or deeper tissues. Surrounding tissues were readily peeled off and no evident blood vessels leading to the tumor mass were seen. The wound was closed without drainage; the postoperative course was uneventful. No evidence of recurrence has appeared up to the present, six months after operation.

#### PATHOLOGIC REPORT

*Gross Description:* The specimen consists of a roughly oval mass of gray-white tissue measuring 5 cm. in length and 2 cm. in thickness. The surface is rough and irregular. Consistence is uniformly firm. The cut section shows a smooth, glistening, gray-white surface with several calcified areas in it.

*Microscopic Examination:* The groundwork of this specimen is made up of a younger and more undifferentiated cell type than that of the previous case. In places there is a small amount of relatively normal, adult connective tissue with narrow cells, and small vesicular nuclei containing rather little chromatin. The collagen is well developed and runs in wavy bundles of fine fibrils. Fibroglia fibrils are present in considerable numbers. The larger part of the tumor is composed of masses of cells of irregular size and shape, usually oval or polygonal. The nuclei are quite large, and they contain a large amount of dense chromatin. The cytoplasm ranges from slightly acidophilic to strongly basophilic. Single and multiple mitoses are frequently seen. There are many tumor giant cells present with multiple large irregular nuclei containing large masses of chromatin. Foreign body giant cells with rounded nuclei of regular shape and size and a uniform cytoplasm are present as well.

Two types of bone formation may be observed in this tumor. The osteoid type seen in the previous specimen is present but is not the predominating picture. In this specimen the atypical fibroblasts are laying down dense homogeneous intercellular substance which finally becomes true bone. In most places in the tumor the fibroblasts are laying down a blue-staining intercellular substance around each cell. As this material increases, the cells change their shape and appearance to take that of true cartilage cells lying in oval or fusiform cartilage spaces. Bundles of homogeneous fibrils penetrate the cartilage from the adjoining osteoid tissue and this fibrillar material becomes calcified to form true bone. As in the

previous tumor, the bone formation has not the regularity seen in bone of normal growth. There are areas present, however, where the bone formation is relatively normal and of adult type. Rows of osteoblasts are ranged around the edges of the spicules of bone and are laying down more hyaline material. The blood vessels are rather few in number and are extremely well formed. The vascular channels lined by tumor cells described by some observers are not seen. There is no evidence of involvement of muscle.

#### DISCUSSION

Pathologists have never agreed as to the nature of the cell which forms bone. Kolodny states that the osteogenic sarcoma is a tumor derived from cells which are descendants of mesoblastic elements predestined embryologically to form bone. As evidence of this he states that metastases from osteogenic sarcoma to regions such as the lung, far distant from the primary tumor, show typical bone formation. Mallory, on the other hand, feels that the osteoblast is simply a fibroblast which under certain conditions of stimulation differentiates to form bone. Bone formation in old inflammatory processes is quoted to prove this. Such an osteoblastic function is occasionally taken on by the fibroblasts of the lung in chronic inflammatory conditions. In refutation of this view the point is made that such bone formation is metaplastic rather than neoplastic. It seems to us that the tumors under discussion present all the histologic characteristics which are required to identify an osteogenic sarcoma, although they had no connection with bone and apparently appeared in response to trauma. To make the point clear a discussion is given of the criteria on which the diagnosis of osteogenic sarcoma is made.

The characteristic cell of the osteogenic sarcoma is of the spindle type, varying from small to large size with a hyperchromatic nucleus and cell borders which are difficult to distinguish. These cells are usually considered to be fibroblasts and when proper staining methods are employed, fibroglia fibrils can be demonstrated. Other cells are present which range from spindle to polyhedral in shape and are extremely variable in size. The nuclei are also variable in size and shape and contain a very large amount of chromatin. These cells may take on the usual outline and polyhedral shape of

bone and cartilage cells and are considered to be the characteristic cells of bone tumors. They often show single and multiple mitoses. Tumor giant cells are formed by multiple mitosis of cells of this type and when found are usually considered to be pathognomonic of sarcoma. The formation of hyaline, osteoid, cartilaginous and osseous intercellular substance is seen in both normal and pathological bone formation. Neoplastic ossification is said to differ from metaplastic ossification chiefly in that in the former the fibrillar base for ossification is not preëxisting, but is formed in the process of ossification by way of cell proliferation. Focal proliferation of tumor spindle cells in rows with fibrillation, hyalinization and finally calcification of intercellular substance are the main stages of neoplastic ossification in osteogenic sarcoma. Intracartilaginous ossification is often seen as well. The arrangement of new formed bone is considered to be trabeculated instead of lamellated as seen in physiologically normal bone. There is one criterion accepted by Kolodny and others with which we cannot agree. That is the presence of vascular channels lined by tumor cells instead of endothelium. These structures we have not been able to identify in proved osteogenic sarcomas.

Histologic examination of these two specimens shows that every one of the criteria for the identification of osteogenic sarcoma has been fulfilled by these tumors. All the variations in morphology shown by tumor cells are present. Multiple mitoses and tumor giant cells may be seen. All the varieties of new bone formation are going on and every stage in the process up to adult bone is present.

From these facts we can only conclude that neoplastic bone formation may take place without connection with primitive or adult bone-forming cells. It would appear that under certain conditions fibroblasts can take on the function of tumor as well as of metaplastic bone formation.

Aside from the theoretical considerations these cases are important because they demonstrate that the bad prognosis ordinarily attached to osteogenic sarcoma does not always hold true when a tumor warranting such a diagnosis histologically is found unattached to bone.

These cases illustrate the fact that tumors may develop in subcutaneous tissue not attached to bone which have a histologic appearance identical with that of osteogenic sarcoma. If these speci-

mens had been removed from a tumor which involved bone the prognosis for the patient would have been extremely grave. In the cases reported there has been no evidence of recurrence two years after operation in one case and six months in the other.

#### SUMMARY

1. Two tumors of soft tissues not attached to bone are described which had a histologic structure identical with osteogenic sarcoma.
2. These tumors have shown no recurrence after local removal.
3. The cases lend support to the theory that the fibroblast in any part of the body may give rise to tumor bone.

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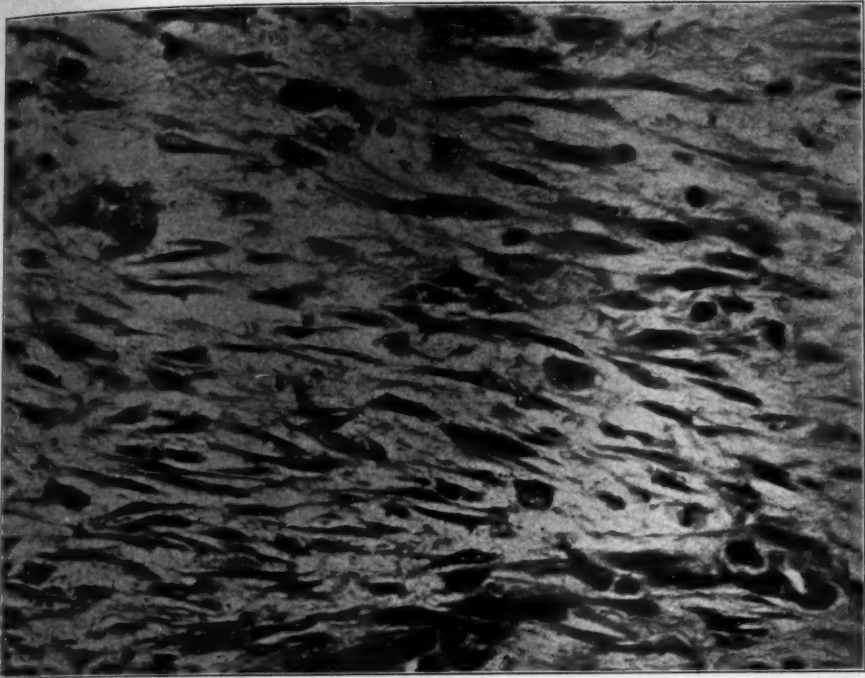
## DESCRIPTION OF PLATES

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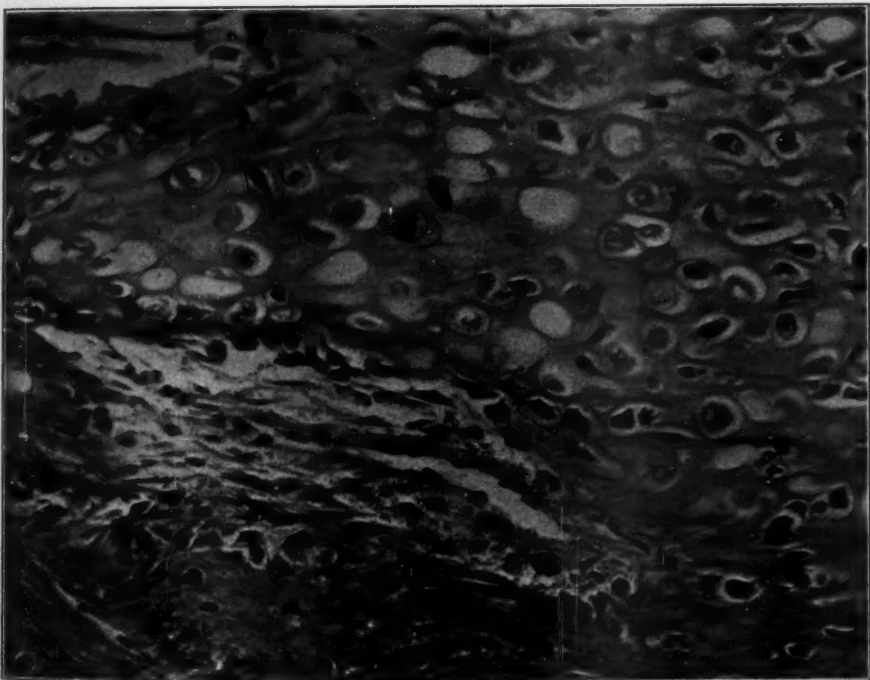
### PLATE 83

FIG. 1. A section from Case I showing three mitotic figures. In this region the tumor is growing like a fibrosarcoma.  $\times 500$ .

FIG. 2. A section from Case I showing cartilage formation and a group of actively growing fibroblasts.  $\times 250$ .



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Rhoads and Blumgart

Osteblastomas Not Connected with Bone

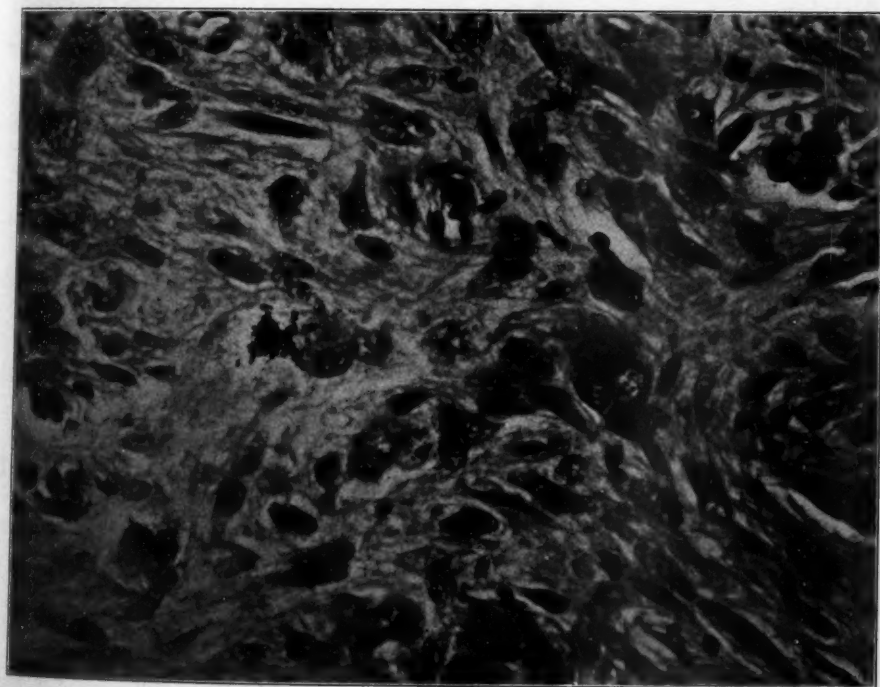
PLATE 84

FIG. 3. Ossification of intercellular hyaline matrix in Case I. Here bone is being formed directly from osteoid tissue without the presence of cartilage.  $\times 100$ .

FIG. 4. A rapidly growing area in Case II showing a multiple mitosis and a multinucleated tumor giant cell. Note the irregularity in size and shape of the nuclei.  $\times 500$ .



3



4

Rhoads and Blumgart

Osteblastomas Not Connected with Bone

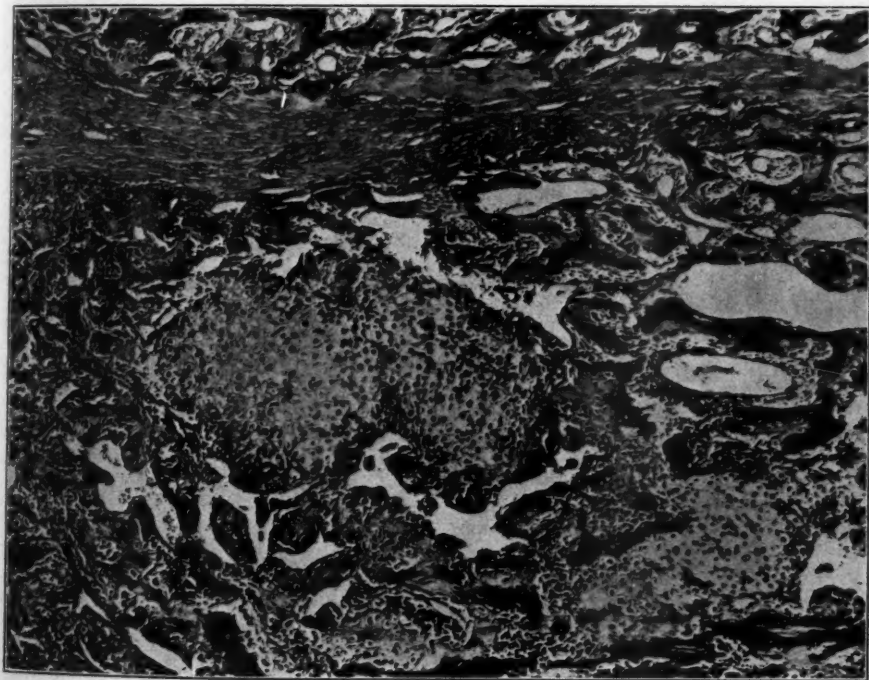
PLATE 85

FIG. 5. Section from the same case showing a tumor giant cell. Here again the irregularity of cell structure is well shown.  $\times 500$ .

FIG. 6. Active bone formation in Case II with calcification of spicules of osteoid tissue.  $\times 80$ .



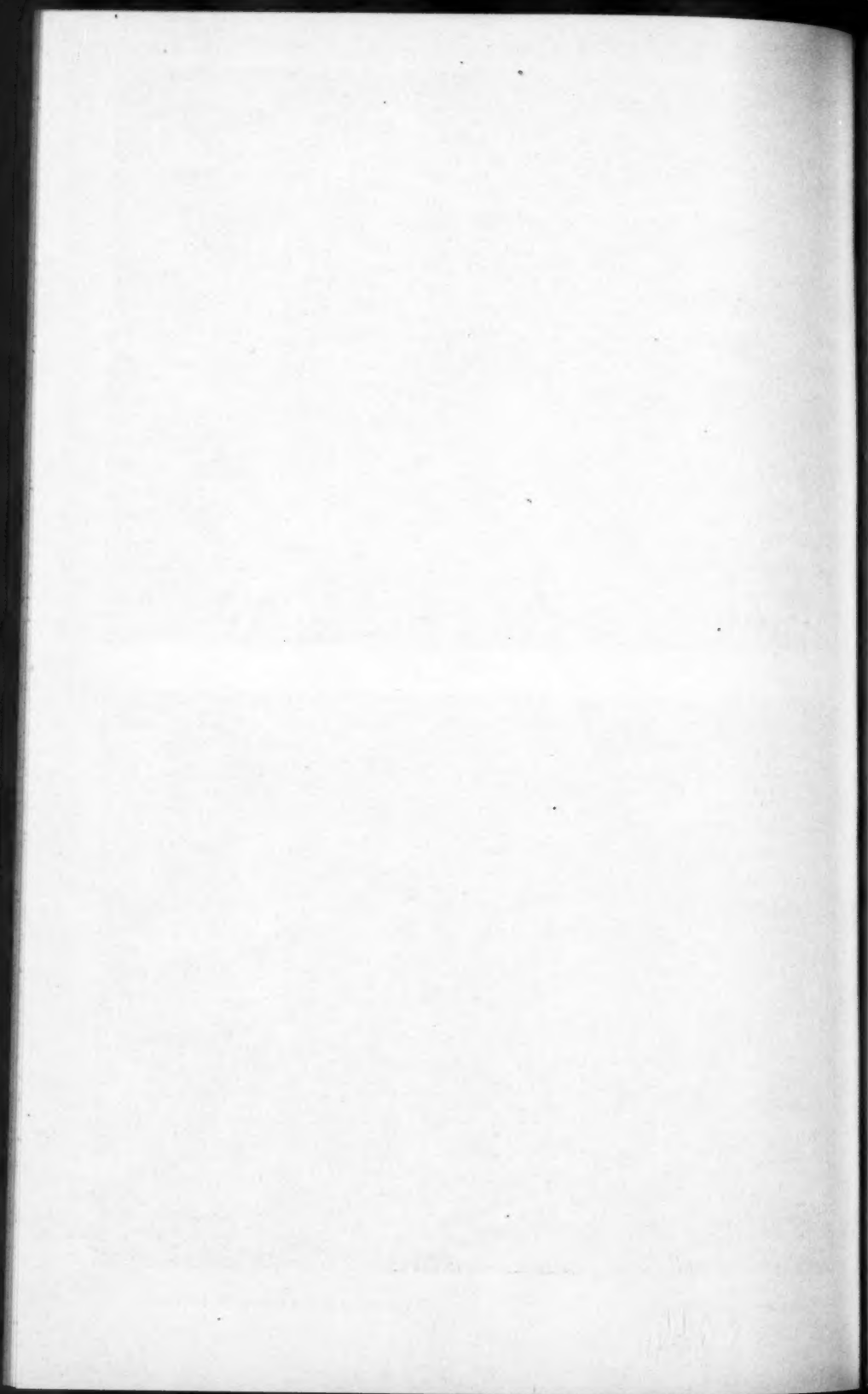
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Rhoads and Blumgart

Osteblastomas Not Connected with Bone



## A DERMOID OF THE CORNEA IN A GUINEA PIG \*

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The purpose of this paper is to place on record a case of dermoid of the cornea observed in an otherwise normal adult female guinea pig. The American Encyclopedia of Ophthalmology<sup>1</sup> states that "primary dermoids of the cornea are extremely rare, most growths involving this structure having their origin in the conjunctiva." Four cases are cited from the literature. In two the tumor was at the limbus, extending over a portion of the cornea and also on to the sclera. In only one of the four cases was histologic study made of the enucleated eye with tumor attached. The latter was the size of a hazel-nut, occupied the entire cornea and encroached upon the sclera. The iris and shrunken, partly calcified lens were adherent to the inner aspect of the cornea.

Fuchs,<sup>2</sup> De Schweinitz,<sup>3</sup> Von Graefe,<sup>4</sup> Greeff,<sup>5</sup> and Strawbridge<sup>6</sup> each report a case, in man, of dermoid at the limbus extending over portions of the cornea and sclera. In spite of the fact that in each instance most of the tumor was over sclera, the respective authors refer to them as corneal dermoids. Greeff mentions two other cases of so-called corneal dermoids in man and one in a dog, that he collected from the literature. Strawbridge quotes Ryba, who collected all cases of dermoid of the cornea published prior to 1853; there were twenty-seven in man, three in oxen, and four in dogs. The bilateral occurrence of dermoids extending over portions of cornea and sclera is reported by Taliaferro<sup>7</sup> and Virchow.<sup>8</sup> Noyes<sup>9</sup> describes three sessile dermoid tumors in the eye of a man "growing upon the limbus corneae equidistant from each other."

### GROSS DESCRIPTION

In the center of the cornea of the guinea pig's right eye is a yellowish, fleshy, round, convex disc bearing a tuft of hair. The diameter of this disc is 5 mm., and in its center at the point of greatest convexity it is 3 mm. thick. There are no dermal or vascular connec-

\* Received for publication May 10, 1928.

tions visible in the gross between the disc and the palpebrae. The sharp line of transition between transparent cornea and opaque disc gives to the latter the appearance of being "stuck on." The tuft of hair is gray, the same color as the fur of the rest of the body. But, whereas the latter is smooth (lying down) the hairs of the tuft stand out perpendicularly to the surface of the disc.

The right eye exhibits no other abnormalities. The distance between the canthi is 1.2 cm. in each eye, but the right eye appears slightly more prominent than the left.

#### MICROSCOPIC STUDY

*Methods:* The guinea pig was killed by etherization. The head was separated from the body and fixed in Kaiserling's solutions. The right eye was dissected out with palpebrae attached, no other anomalies being noted. It was then embedded in celloidin in the usual manner and sections approximately 20 microns in thickness were cut for study. The stains used were hematoxylin and eosin.

*Microscopic Findings:* The cross-section of the tumor is roughly a half-circle, convex surface externally. It is composed mainly of fat tissue and rests directly upon substantia propria corneae. It is covered with stratified squamous epithelium whose basal layer contains pigment, and beneath which is a layer composed of interlacing bundles of fibroblasts containing sebaceous glands and arrectores pilorum muscles. This layer corresponds to the corium of the normal integument. Passing through this layer or corium are hairs whose bulbs are embedded in the areolar tissue beneath. In the corium and fat tissue are capillaries, venules and small arterioles.

On each side of the tumor, cornea and conjunctiva are normal. At the margin of the tumor, the layer of stratified squamous epithelium that covers it becomes continuous with the conjunctiva. In this region also the substantia propria corneae splits into two portions. An external, smaller portion becomes continuous with the layer of the corium of the tumor, and the larger inner portion continues under the tumor forming the base upon which it rests. The anterior basal lamina of the cornea is clearly defined in the normal portions but may be followed only a short distance over the edge of the tumor. Here it rapidly loses its identity, becoming continuous with that part of the corium immediately beneath the layer of

stratified squamous epithelium. In the marginal regions of the tumor are also anastomoses between capillaries of the corium and of the conjunctiva. A large venule and an arteriole are seen passing between the corium of the tumor and the subconjunctival tissue.

#### DISCUSSION

Concerning the origin of corneal dermoids nothing can be added to what has been already said in regard to the origin of dermoids in general. The occurrence of true corneal dermoids, as previously mentioned, is very rare, and in the opinion of the writer still less frequent than the literature would indicate. For, as pointed out, not only were a number of dermoids, reported as corneal in origin, situated at the limbus with more of the tumor over sclera than cornea, but microscopic study of them *in situ* was not made. Their classification, therefore, as true corneal dermoids is not justified. Noyes<sup>9</sup> and Alt<sup>10</sup> regard all dermoids at the corneo-scleral junction as conjunctival in origin. This view is probably correct.

The following evidence indicates that the dermoid described in this paper is corneal and not conjunctival in origin:

1. The entire base of the tumor rests upon substantia propria corneae, nothing intervening.
2. The substantia propria corneae splits at the margin of the tumor, one part becoming continuous with a part of its superficial wall, the other passing beneath it.
3. As a corollary to the preceding observation, the layer of substantia propria corneae beneath the tumor is narrower than it is at the sides of the tumor (*i.e.*, before it splits).
4. The anterior basal lamina of the cornea extends into the tumor becoming continuous with the most superficial portion of the layer of corium.

The foregoing facts are irreconcilable with a conjunctival origin. If this were the case a dermoid of this size would rest upon the cornea, not in it. The cornea would not split at the margins of the tumor to include it, as it were. The anterior basal lamina would pass beneath the tumor and not become continuous with its most superficial part. Or if there was secondary invasion of the cornea, the anterior basal lamina would be traced into the basal regions of the tumor.

## SUMMARY

1. A case of true dermoid of the cornea in a guinea pig is recorded.
2. All dermoids reported as corneal in origin cannot be finally classified as such because of lack of microscopic evidence and because many of them were at the limbus with more of the tumor extending over sclera than cornea.

The author wishes to thank Miss Lillian M. Leavitt for the preparation of the sections and Dr. F. B. Mallory and Miss Catherine G. Norton for the illustrations.

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## DESCRIPTION OF PLATES

## PLATE 86

FIG. 1. Guinea pig showing erect position of hairs of right corneal tumor.

FIG. 2. Head of guinea pig after separation from body and fixation in Kaiserling's solutions. The eye has been rotated superiorly and anteriorly to show lateral view of the corneal tumor. Note "stuck on" appearance of tumor. Some of the hairs have dropped off.



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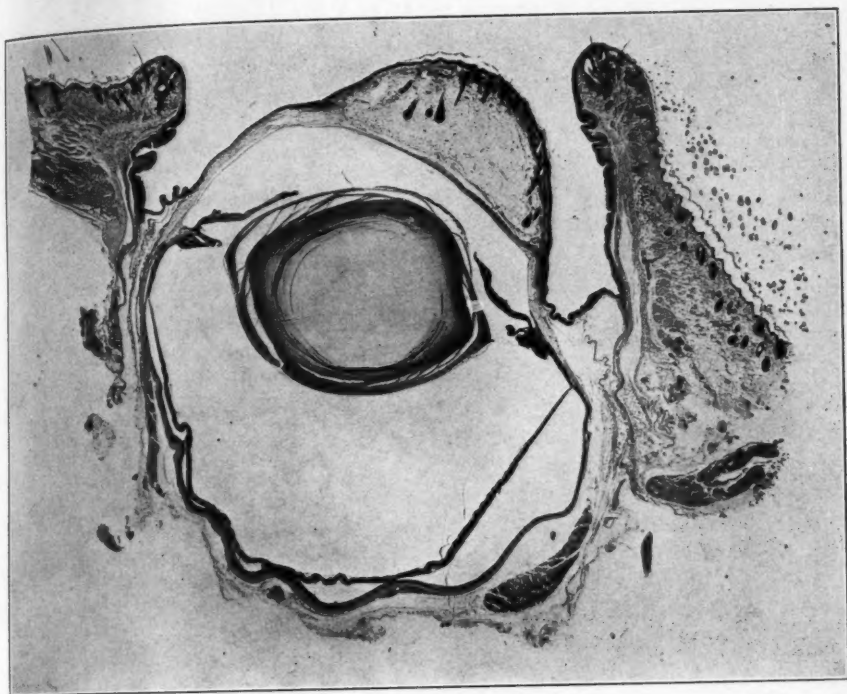
Brunschwig

Dermoid of Cornea in a Guinea Pig

PLATE 87

FIG. 3. Sagittal section through eye, tumor, and palpebrae, approximately in midline. Some distortion due to fixation. During life tumor was in center of cornea. Palpebrae, lens, ciliary body, wall of bulbous oculi are normal.  $\times 8$ .

FIG. 4. Enlargement of tumor seen in Fig. 3. Sagittal section through midline of tumor, which is composed mainly of areolar tissue, covered externally by stratified squamous epithelium. Beneath the latter is layer corresponding to corium of normal integument, and containing sebaceous glands and arrectores pilorum muscles. Bulbs of hair in areolar issue. Hair shafts passing through corium. Tumor rests upon substantia propria corneae. At margin of tumor stratified squamous epithelium becomes continuous with conjunctiva. Substantia propria corneae splits into two portions. The smaller external portion is continuous with layer of corium, the internal larger portion passes beneath tumor.  $\times 15$ .



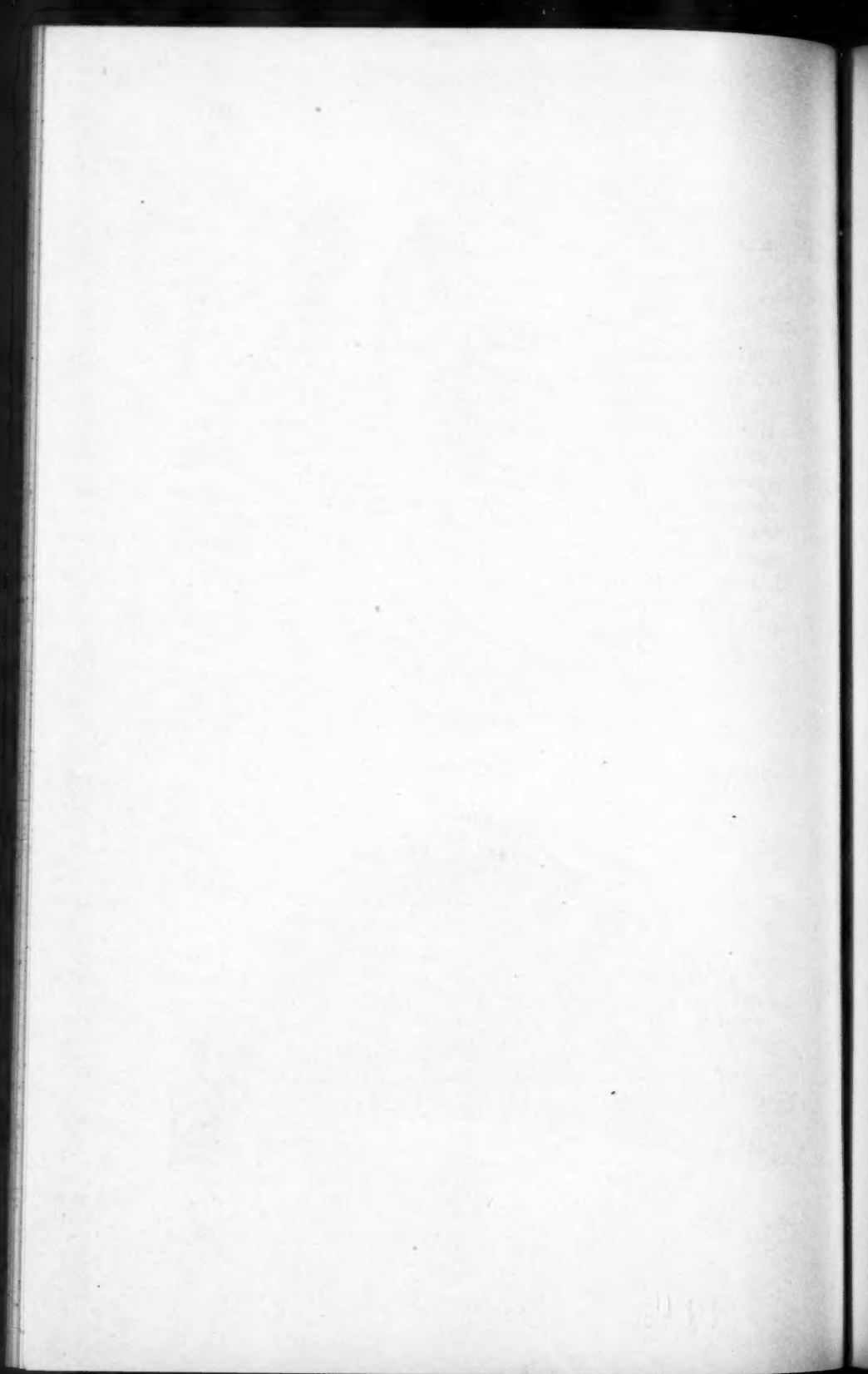
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Brunschwig

Dermoid of Cornea in a Guinea Pig



## OBSERVATIONS ON INCUBATED TISSUES AND EXUDATES \*

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In two recently published articles the authors have described certain cells which develop in incubated leukemic and normal blood.<sup>1, 2</sup> The appearance and activity of these cells under various abnormal conditions were discussed in a third paper.<sup>3</sup> The cells which were seen in these experiments were so striking and characteristic in their appearance that we thought it a matter of interest to determine whether or not the same types of cells would develop in tissues kept under similar conditions. We felt that from a study of tissues it might be possible to determine what cells are the progenitors of the large cells of the blood.

In the previous papers we have defined the various terms used. It is sufficient to say here that we follow essentially the nomenclature used by Sabin, Doan and Cunningham.<sup>4</sup>

### LITERATURE

Although a large amount of work has been done on tissue cultures little attention has been paid to the classification and description of the wandering phagocytic cells which develop in them.

Lewis, Willis and Lewis<sup>5</sup> used plasma clot, coverslip preparations of tuberculous tissue and watched the development of epithelioid cells. They thought that such cells arose from either monocytes or clasmatocytes and felt that they could see transition stages between both these types and epithelioid cells.

Lewis and Webster<sup>6, 7</sup> described experiments in which they cultured tissue from normal, and acutely and chronically inflamed lymph nodes. Nodes of Hodgkin's disease, tuberculosis, carcinoma, melanotic sarcoma, and lymphatic and myelogenous leukemia were also used. Giant cells developed in all of these cultures, which when stained supravitaly showed a central red zone and peripherally arranged fat globules. They described the appearance of a wander-

\* Received for publication April 14, 1928.

ing cell which migrated out of the tissue during the first few hours of incubation, kept moving for forty-eight hours and then became quiescent and died. A second type was seen which was called the endothelial type and which contained a well marked rosette stained with neutral red. All transitions could be seen between wandering cells and endothelial cells, and between endothelial cells and giant cells. The endothelial cells did not begin to migrate from the tissue until after twenty-four to forty-eight hours of incubation. They were cells of large size, irregular outline, and rather sluggish motility. Forms simulating wandering cells and fibroblasts were seen. These workers never saw transition stages between lymphocytes and the wandering or endothelial types of cell.

Maximow<sup>8</sup> cultivated rabbit mesenteric lymph nodes, omentum and loose connective tissue. The preparations were made in plasma clots on coverslips, and bone marrow extract was used to supply the required growth-stimulating substance. The cultures were inoculated with human tubercle bacilli in order to watch the development of tubercles *in vitro*. The large, pale cells of the lymph nodes, including those lining lymph sinuses, were seen to proliferate and become ameboid and phagocytic, sometimes epithelioid in type. They stored dye and formed giant cells. Lymphocytes were also seen to change in appearance, enlarge, proliferate and to become ameboid and phagocytic. Maximow believes that a large group of primitive cells, mesenchymal in type, exist such as the clasmatoocytes of the tissues, the reticular cells and cells lining the sinuses of lymph nodes, spleen and bone marrow, and the Kupffer cells of the liver sinusoids. He holds that these cells are pluripotent and that when stimulated by the use of various dyes and colloidal materials they may become actively phagocytic. He feels that under other conditions they may give rise to lymphocytes and even to granulocytes.

Carrel and Ebeling,<sup>9, 10, 11</sup> studied a long series of cultures in which both tissue and blood cells were examined. They found that cultures in serum allowed the growth of mononuclear cells only, whereas in plasma clots both fibroblasts and mononuclear cells grew out. They describe the transformation of monocytes into clasmatoocytes and also into fibroblasts.

McJunkin<sup>12</sup> described observations on lymph nodes and on peritoneal exudates of rabbits, although no cultures were made. He saw both "rosette" and "non-rosette" cells in normal rabbit lymph

nodes. By "rosette cell" he meant a cell with a considerable clump of fine red granules in the indentation of the nucleus. He stated that all transitions were present from the "reticular" cells with a single small collection of fine granules to those with rosettes and numerous scattered red granules. From his experiments he concluded that the blood monocyte arose in lymph nodes and that the larger cells with irregular neutral red bodies were derived in part from the blood monocytes and in part from vascular endothelium.

Sabin holds that no monocytes are found in rabbit nodes and that large numbers are present in the spleen. She considers that they arise from a primitive reticular cell which exists in spleen, liver, lung and the milk spots of the omentum. The clasmatoocyte according to her theory arises from fixed endothelium of the type found lining the sinusoids of the liver or the lymph channels of the spleen and lymph nodes. She feels that the monocytes and clasmatoocytes are quite distinct types and that one never becomes the other.

Sugiyama<sup>13</sup> and other embryologists state that while cells of the clasmatoocyte type may be seen in chick embryos after the first few days of incubation, monocytes do not appear until about the second week. This point is of great importance in proving that the two types of cells are of a different origin.

#### CULTURES OF EMBRYONIC TISSUE

In our experiments, a series of chick embryos of various ages up to ten days were cut in small pieces and suspended in tubes of serum. They were incubated at 37.5° C and control tubes were kept in the icebox. Bits of tissue were removed with a platinum loop and mounted on neutral red slides for supravital observations. The only motile phagocytic cells seen before incubation were clasmatoocytes.

At the end of twenty-four hours a fair number of cells had appeared. Most were of the clasmatoocyte type and contained many neutral red granules of irregular size and shape. The cell outline was often indented or fusiform. A few cells were seen which showed a rosette arrangement of fine red granules in the center of the cell. Outside this was a row of regular refractile granules. These cells were less actively motile and showed a more uniform outline than those of the clasmatoocyte type. They were considered to be monocytes. Cells of these same types persisted for several days until the death of the cultures.

Rabbit embryos, eighteen days old, were used in a somewhat similar experiment. Both coverslip, plasma clot preparations and bits of tissue suspended in serum were set up. Embryonic liver and subcutaneous tissue were used. No monocytes were noted in spreads of the tissues made before incubation. At the end of twenty-four hours a number of cells were seen which had a fairly regular outline, a well marked focus of fine neutral red granules, an indented nucleus and a peripherally arranged row of refractile granules. A second type of cell was found which had a more irregular outline, a large number of red granules of various sizes and depth of color and rather few refractile granules. At the end of forty-eight hours incubation the same types of cells were present and were considerably larger than before. At the end of three days the refractile granules were very marked in the rosette type. The rosette was of good size and composed of rather finely divided masses of neutral red. The second type showed far fewer refractile granules and more variable masses of neutral red though a rosette of fine granules was often present. The cells were very irregular in shape and many elongated forms were seen. At the end of four days incubation the picture was essentially the same and after six days the cells were all dead.

From these experiments we may conclude that clot and serum cultures of chick and rabbit embryos show the same changes in the wandering cells as are seen in incubated adult human and rabbit blood. Rosette and non-rosette cells develop, hypertrophy and show refractile granules, more in the rosette than in the non-rosette type.

#### EXUDATES

The formation of exudates in the pleural cavities of guinea pigs was induced by the injection of 5 cc. of ordinary bacteriologic beef infusion broth containing 0.1 per cent of dextrose. The exudate was withdrawn after twenty-four hours and examined supravitaly. Two types of cells could be seen besides lymphocytes and granulocytes. One was of large size and irregular outline. The cytoplasm was packed with variously sized masses of neutral red. Phagocytosed erythrocytes and polymorphonuclear cells were seen. The second type was smaller and tended to be round. A focus of fine neutral red granules was present near the nucleus. The cytoplasm contained a large number of refractile granules arranged peripherally. Only a moderate amount of phagocytosis was seen in this type of cell.

Exudates were produced in rabbits by the intrapleural injection of 5 cc. of the animal's own blood removed by cardiac puncture and injected before the blood clotted. Specimens were withdrawn at various periods and observed in supravital spreads and in fixed preparations. After twenty-four hours the cells were almost identical with those seen in the tubes of blood after several days incubation. It was possible to identify two types of cells. One showed a definite, rounded focus of fine neutral red granules surrounded by a peripheral zone of unstained refractile granules. This cell was usually from ten to forty microns in diameter and had quite a regular outline. There was some phagocytosis of red cells. The second type was a larger cell of more irregular shape which tended to be more actively motile. This type of cell had a cytoplasm almost filled with red-staining masses of phagocytosed material. In the dried preparations stained by Wright's method the first type of cell showed a round to oval nucleus with rather light staining chromatin. The cytoplasm was often entirely filled with unstained vacuoles. The second type of cell had one or more round to slightly oval nuclei containing fairly dense chromatin. The cytoplasm was a fairly uniform blue in color and contained irregular masses of red-staining material. Sometimes a few basophilic granules were present.

Ascitic fluid was removed from a patient who had been ill with chronic lymphatic leukemia for several years. Fixed and supravital preparations of the fluid showed it to contain a large number of typical small lymphocytes, none showing more than one to two granules of neutral red. No monocytes were seen in counting four hundred cells. Tubes of this fluid were incubated at 37.5° C and observations were made every two days. On the sixth day cells appeared which were many times the size of a lymphocyte. They had an indented nucleus often containing one or more nucleoli. A few refractile granules were present. Rather fine and diffusely scattered neutral red granules were seen, usually collected in a focus in the cytoplasm. This type of cell was extremely phagocytic, often containing large masses of red-staining material or carbon in the cytoplasm. In the Wright's stained preparations a large number of typical "X" cells were found. The nucleus was oval or sometimes indented. Many multinucleated forms were present. There was a large amount of evenly stained blue cytoplasm which often contained masses of red-staining material or small basophilic granules.

Phagocytized carbon granules were often present. The oxidase reaction was both positive and negative. These cells remained alive for several days and then all died.

The results of this experiment seem to bear out the work of Bloom.<sup>14</sup> This investigator made cultures of clotted lymph obtained from the thoracic duct. The lymphocytes originally present proliferated, hypertrophied and became ameboid and phagocytic. Observed in supravital spreads these cells were quite typical monocytes with well formed rosettes. From these results he argued that monocytes took their origin from lymphocytes and were simply one type of the polyblast of Maximow.

#### ANIMAL TISSUES

A series of cultures of rabbit lung, spleen, liver, lymph node, bone marrow and subcutaneous tissue were examined. The tissue was cut into small pieces and several bits placed in test tubes containing about 0.5 cc. of homologous serum. Supravital and stained preparations were made at frequent intervals and the changes in the cells observed. Before incubation cells of both the clasmatocyte and monocyte type were present in all tissues examined except the lymph nodes. Here only clasmatocytes were seen.

At the end of twenty-four hours incubation only a few cells had appeared. These were of both the rosette and non-rosette type. The size was about that of the ordinary monocyte and no refractile granules were present.

After forty-eight hours all the cultures showed a good growth of cells and two types were seen. One had a well marked rosette, often of the hypertrophied type, composed of many fine red granules, little phagocytized material and few refractile granules. The second type contained a smaller focus of neutral red granules close to the nucleus and a large number of refractile granules.

As the period of incubation went on the first type of cell containing the large rosette of fine granules enlarged greatly and became very actively phagocytic. The second type did not increase so much in size, preserved a definite, though often very small rosette, and exhibited a large number of refractile granules in the cytoplasm.

A similar series of observations were made on a group of animals which had received repeated injections of streptococci. These ani-

mals showed a very high count of monocytes in the blood. The organs such as the lung, spleen, liver and bone marrow were found to contain a large number of these cells with hypertrophied rosettes. Cultures of these organs showed a large number of cells with variably sized neutral red rosettes and many refractile granules. Frequently these cells grew to tremendous size but preserved the same general appearance. A very limited number of cells with irregular outline, ill-defined rosettes and active phagocytosis were seen.

From this experiment it is possible to conclude that the monocyte is the progenitor of the rosette, refractile granule type of cell which we have called a "Y" cell in previous studies.

#### HUMAN LYMPH NODES

A series of human lymph nodes were examined by a technique similar to that used with the animal tissues. The specimens also were all run through the routine histologic technique of paraffin embedding, sectioning and staining with eosin-methylene blue. Several showed only chronic inflammation, one contained metastatic fibrosarcoma and two were from typical cases of Hodgkin's disease. The tissue was brought aseptically from the operation, cut in small pieces and suspended in either sheep serum or ascitic fluid. It was known from previous observations that both of these fluids made good media for the development of phagocytic cells. Supravital and fixed observations were made on bits of tissue before incubation. After the preparations were set up pieces of tissue were fished out of the medium with a platinum loop and observations were made on both supravital spreads and on smears stained by Wright's method and by the oxidase reaction. To make the discussion of the findings clear it is necessary to define the terms used.

"*Reticular*" cell refers to a group of cells as large or larger than a blood monocyte. The nucleus contains a rather small amount of chromatin and is of good size, sometimes indented and often lobed. The amount of cytoplasm is often variable. Some of the cells have little more cytoplasm than a large lymphocyte and others have quite a large amount. There is a focus of rather fine, uniform, neutral red granules closely related to the indentation of the nucleus and often occupying nearly half of the total cell cytoplasm. Unstained refractile granules are very few.

*Lymphoblast* refers to a cell from ten to fourteen microns in diameter. The nucleus is round, of good size, and has a peculiar ground glass appearance when examined vitally. There is rather little cytoplasm as compared with the size of the nucleus and few or no neutral red granules are seen.

The "X" cell is a cell varying in size from that of a large lymphocyte up to a form somewhat larger than the reticular cell and has a rather irregular outline. The nucleus is round or oval and more than one nucleus may be present. The cytoplasm is variable in amount and contains a large number of fine, uniformly sized neutral red granules scattered irregularly throughout. Phagocytosis may be quite active in this type of cell and sometimes the cytoplasm is almost filled with engulfed material. In fixed preparations the nuclei are usually rounded and contain a moderate amount of chromatin. There is a large amount of even blue cytoplasm often containing red-staining masses of phagocytosed cellular material, carbon or fine basophilic granules.

The "Y" cell, as previously described, is a cell seen in incubated blood, which has a round or oval nucleus, a group or a small rosette of rather coarse neutral red particles close to the nucleus and the cytoplasm filled with refractile granules. Phagocytosis is not marked and the cell outline is round and uniform.

Before incubation all the specimens showed four types of cells.

1. Small lymphocytes. These cells had round nucleus and a small amount of cytoplasm containing a few small granules of neutral red.
2. Cells varying in size from slightly larger than a small lymphocyte to a cell twenty or thirty microns in diameter. The nuclei varied from round to oval or indented. In the cytoplasm in close relation to the nucleus there was a rounded focus of fine neutral red granules often similar to the hypertrophied rosette described by other workers.
3. A variable number of cells morphologically similar to human blood monocytes. These cells were from ten to fourteen microns in diameter, contained an indented nucleus and a small unstained area near the indentation around which a few neutral red granules were grouped.
4. Fibroblasts and fixed endothelial cells were seen which contained scattered fine red granules at either end of the nucleus.

The nodes of Hodgkin's disease contained cells unlike those seen in any other condition. These cells ranged from twenty to forty microns in diameter and were rounded and regular in outline. The nucleus was round and very large, occupying a good part of the cell. In the nucleus were one or more large round nucleoli. These cells took no neutral red.

After twenty-four to forty-eight hours incubation the cultures showed a very striking picture. Examined in supravital spreads great masses of cells of one type were seen which varied in their size and content of neutral red granules. Every conceivable stage of rosette formation was present from a few red granules to a mass of fine red particles which filled a large part of the cytoplasm. There were a number of unstained refractile granules. Stained by Wright's method they tended to have an oval or indented nucleus and a variable amount of blue cytoplasm. These were considered to be reticular cells.

A second type of cell was present which was somewhat larger and more irregular in outline than the cell just described. This type showed a large number of irregular red particles scattered without arrangement through the cytoplasm. The outline of the cell was likely to be stellate or elongated. Many of this type were motile. There was often a considerable amount of phagocytosed material in the cytoplasm. This type of cell is identical with the "X" cells seen in incubated blood.

Fair numbers of small lymphocytes were scattered among the large cells. These tended to have more red granules in the cytoplasm than the cells of that type seen in the fresh preparations.

The oxidase stain on smears of tissue before incubation showed in the Hodgkin's cases a few oxidase-positive mononuclear cells in the fixed preparations, which resembled blood monocytes very closely. In the nodes of Hodgkin's disease a very large number of cells were oxidase-positive at the end of forty-eight hours incubation. From that time on the number of oxidase-positive cells decreased until few or none were seen at the sixth or eighth day of incubation.

Two main points of difference were seen between cultures of Hodgkin's disease lymph nodes and the simply inflammatory nodes. One was the presence of the large cell previously described in the Hodgkin's disease cases. The second was the occurrence of many oxidase-positive as well as negative "X" type cells in Hodgkin's

while the "X" cells in preparations from inflammatory nodes were all oxidase-negative.

From the second day of incubation on, the only changes in the cultures were the gradual enlargement of the reticular cells, the appearance of more "X" and "Y" type cells, and the disappearance of the oxidase-positive cells in the Hodgkin's disease cases. The average length of life of the cells was about six days but in a few instances they lived as long as ten or twelve days. As the cultures became old the cells lost their characteristic morphology and became filled with large, irregular masses of hemoglobin.

As previously described, two types of cells which we have called "X" and "Y" develop in incubated blood. In observations on lymph nodes similarly incubated cells of exactly the same morphology, staining properties and physiologic activities as those seen in incubated blood have been observed. In addition a third type of cell appears in the lymph node cultures which we have called a "reticular" cell. The oxidase reaction of this cell may be either positive or negative. Every stage in the transition between "reticular" cells and "X" cells may be seen. Monocytes similar to those seen in the blood are found in certain cases, and "Y" type cells, which we suppose come from monocytes, develop in these cultures.

#### SUMMARY AND DISCUSSION

Tissues from chick and rabbit embryos of various ages were incubated in rabbit serum. Observations made on the tissues before incubation showed only clasmotocytes and no monocytes. After twenty-four hours incubation, cells of the monocyte type with definite neutral red rosettes appeared. This observation would argue that some predecessor of the monocyte is present in early embryonic tissue. There is enough similarity between the wandering phagocytic cells of the clasmotocyte type and the monocytes to make it conceivable that one gives rise to the other.

Pleural exudates induced in rabbits and guinea pigs were examined. Cells were seen which were almost identical with the cells appearing in incubated blood. This is some proof that the cells seen in the blood cultures were not degeneration forms. If the cells of the exudates were suspended in serum they would remain alive for considerable periods.

Ascitic fluid from a case of chronic lymphatic leukemia was found to be almost a pure suspension of cells of the type of small lymphocytes. This fluid was incubated and about the sixth day a large number of good sized, actively phagocytic cells, sometimes multinucleated, appeared which were very much like the "X" type of cell found in incubated blood. Unfortunately, part of the tubes were spoiled and repeated observations to watch transition stages could not be made. This experiment corresponds to the work of Bloom, who cultivated large phagocytic cells from the lymph of the thoracic duct. Such lymph has been shown to contain only lymphocytes.

Pieces of spleen, bone marrow, lung, lymph node and subcutaneous tissue of rabbits were examined supravitaly and then incubated in sheep serum. Cells of both clasmatoocyte and monocyte types were present in all the tissues examined before incubation except the lymph nodes where only clasmatoocytes were seen. After incubation, however, large cells appeared in all the tissues, which were identical with those seen in incubated blood. In tissues from animals in which a monocytosis had been induced before death a larger number of cells of the rosette type with refractile granules were seen in the cultures.

The most interesting experiments were those on human lymph nodes. These are of particular importance in view of the observations of Sabin that monocytes are not present in normal nodes and of McJunkin that the monocyte is the cell involved in Hodgkin's disease. The incubated specimens showed all transitions between cells about the size of a lymphocyte which contained a small number of neutral red granules grouped in a rosette up to very large cells with one or more nuclei and a rounded mass of neutral red granules identical in morphology with the hypertrophied rosette seen in monocytes in tuberculosis. The number of these cells which appeared during incubation was apparently directly proportional to the amount of inflammatory reaction present in the node to begin with and had no relation to the amount or type of tumor present. The cells were phagocytic, often motile, and contained a variable number of refractile granules. In the nodes of Hodgkin's disease many of these cells were oxidase-positive for a period.

We are indebted to Dr. F. B. Mallory and Miss Catherine Norton for the photomicrographs.

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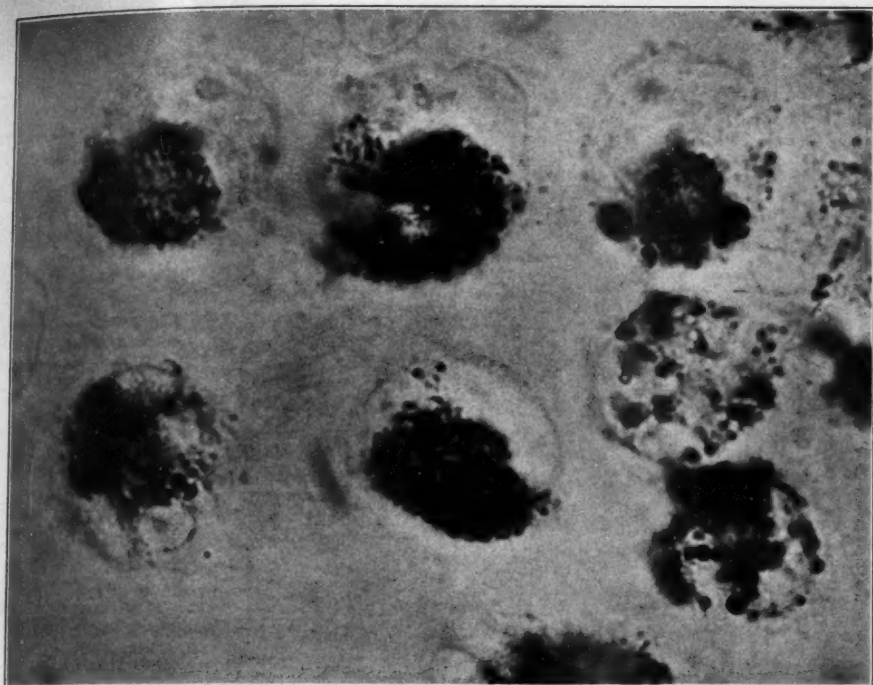
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## DESCRIPTION OF PLATES

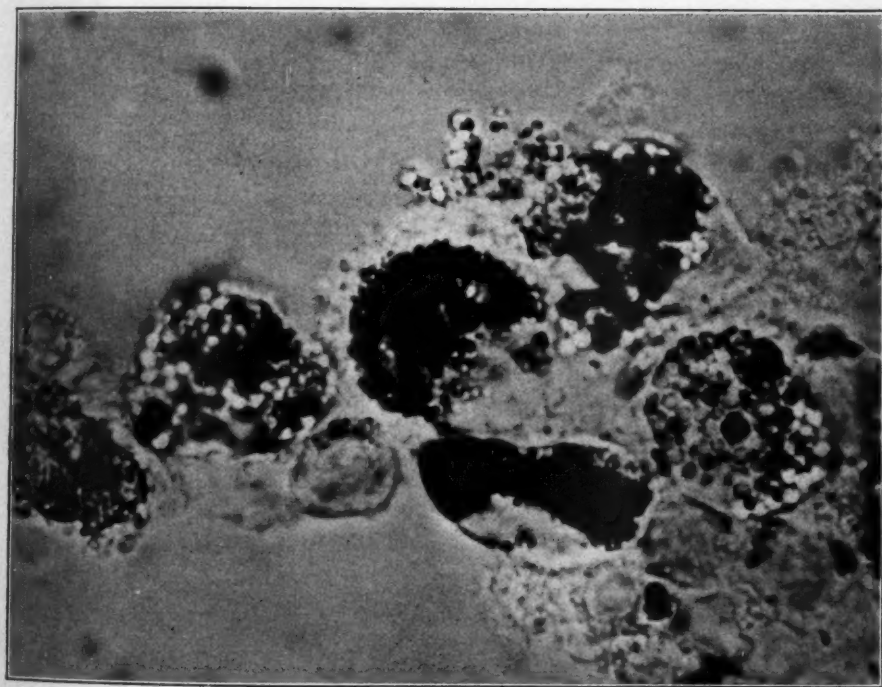
## PLATE 88

FIG. 1. A group of "reticular" cells from a chronic inflammatory lymph node incubated seven days. There are five typical "reticular" cells with well marked deeply stained rosettes. The cell in the upper right corner in addition shows a phagocytosed particle at the periphery of the rosette. The rosettes as can be seen are composed of many fine granules of approximately equal size and occur in the hofs of the indented nuclei. The two entire cells in the lower left corner are not characteristic enough to classify. A supravital preparation.  $\times 2000$ .

FIG. 2. A clump of cells from a Hodgkin's disease lymph node, incubated seven days. Three of the cells are "Y" cells. The neutral red in them occurs in granules and globules of unequal size and with an uneven distribution. Note the large unstained refractile granules characteristic of this type of cell. The small cell near the center is a lymphocyte showing a few fine neutral red granules. Above it to the right is an actively phagocytic "X" cell with a few fine unstained refractile granules. The clear portion of the cytoplasm at the periphery of the rosette can be seen. The elongated cell below this is another "X" cell containing masses of darkly stained hemoglobin. A supravital preparation.  $\times 2000$ .



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Rhoads and Parker

Incubated Tissues and Exudates

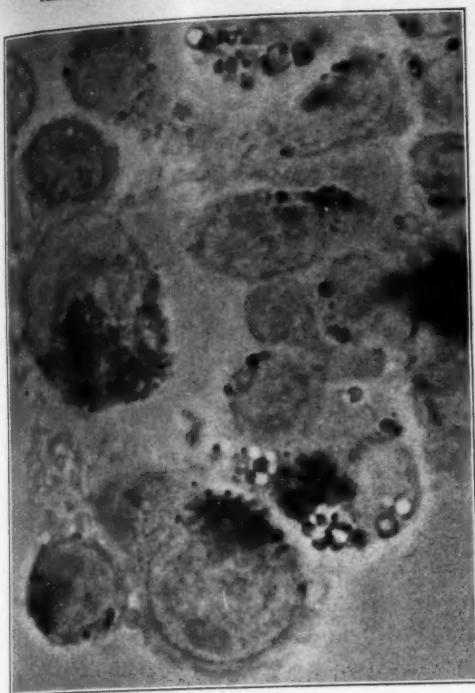
PLATE 89

FIG. 3. Cells from a chronic inflammatory lymph node incubated five days. The second cell from the left at the bottom with the large round nucleus and prominent nucleoli is a young "reticular" cell; it shows a small rosette at one side of the nucleus. Above it, to both the left and right are older "reticular" cells with indented nuclei and well defined rosettes. The remainder of the cells are typical small lymphocytes. A supravital preparation.  $\times 2000$ .

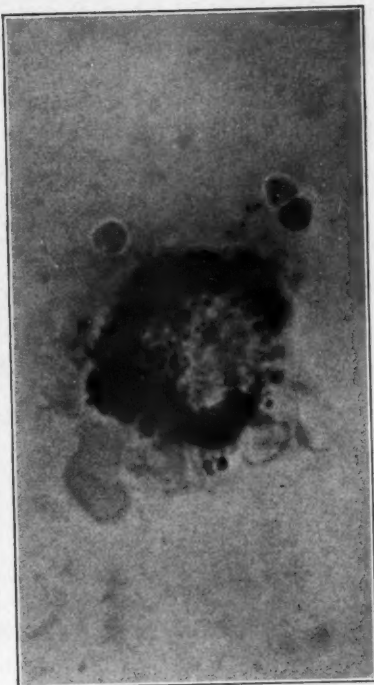
FIG. 4. An "X" cell from an incubated chronic inflammatory lymph node. It contains many large neutral red granules and globules around its poorly defined rosette. The border is irregular due to the numerous pseudopods. A supravital preparation.  $\times 1000$ .

FIG. 5. A clump of cells from a lymphoblastoma lymph node incubated two days. The cell at the center is an "X" with large amount of cytoplasm and eccentric nucleus. Several of the other cells have a considerable amount of cytoplasm and are irregular in outline but are smaller than the "X" cell; these are "reticular" cells. In addition there are a few lymphocytes. The two vacuolated structures are nuclei of dead cells. Wright's stain.  $\times 1000$ .

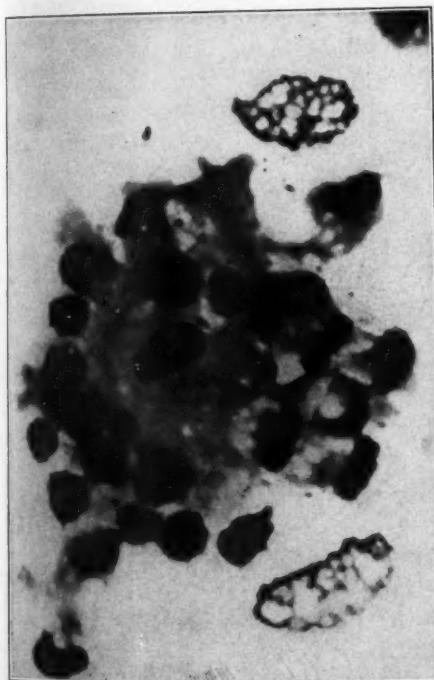
FIG. 6. A binucleated "X" cell from ascitic fluid of a case of chronic lymphatic leukemia, after six days incubation. There are several phagocytosed particles in the cytoplasm. The cytoplasm also shows a considerable number of fine granules and a darker area to the left of the nuclei suggesting the rosette area. Wright's stain.  $\times 1000$ .



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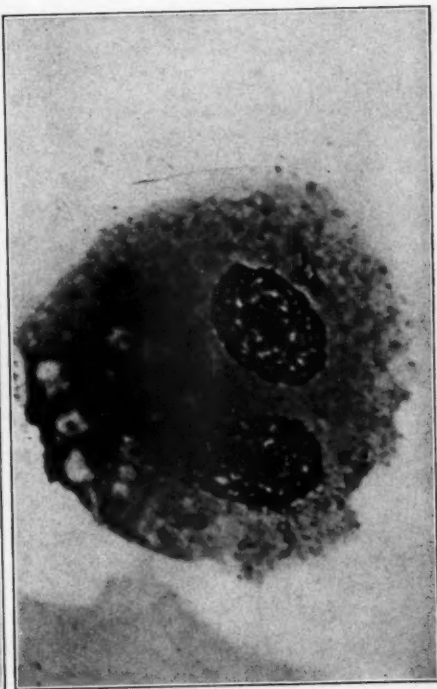


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Rhoads and Parker



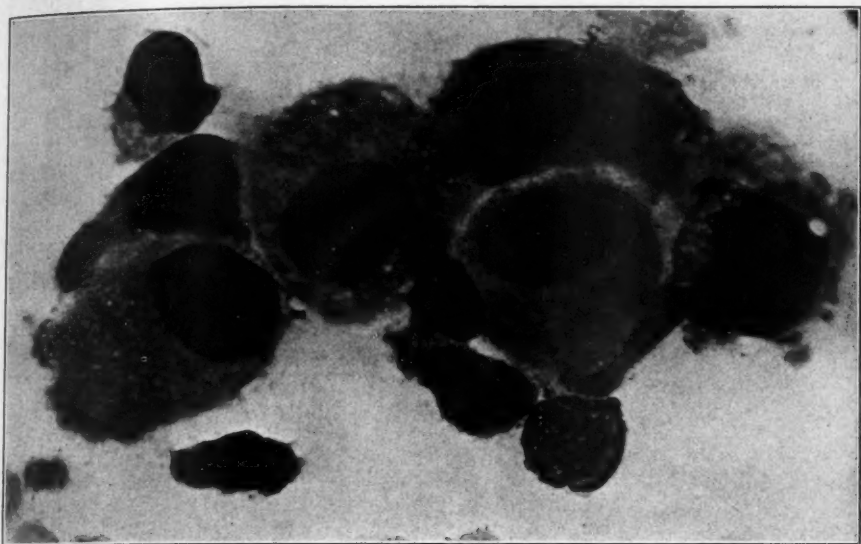
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Incubated Tissues and Exudates

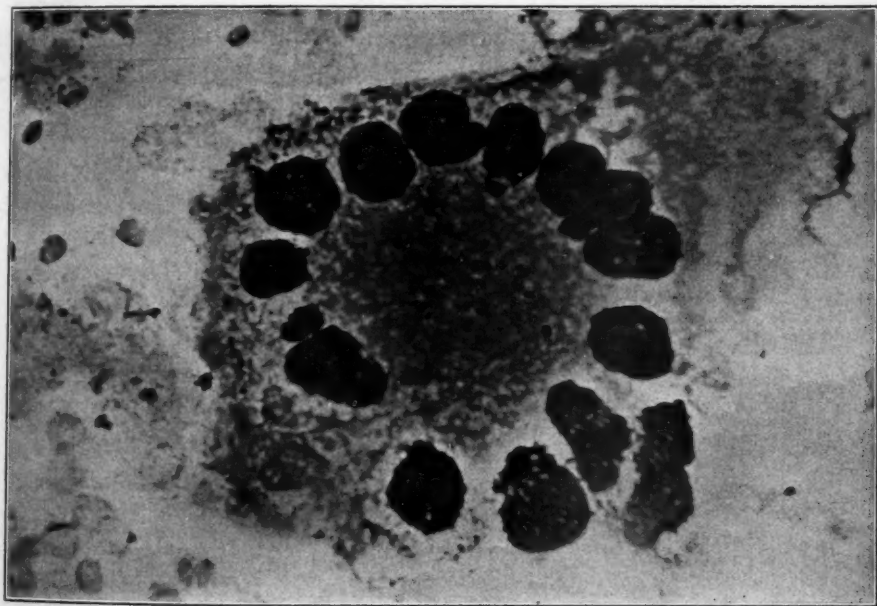
PLATE 90

FIG. 7. A clump of cells from the same preparation as in Fig. 6. The four large cells are typical "X" cells. Granules and fine vacuoles can be distinguished in their cytoplasm. The small cell in the lower left corner is a lymphocyte. The two medium sized cells in the upper left corner are "reticular" cells. The cell at the extreme right suggests an intermediate stage between a "reticular" and an "X" cell. Wright's stain.  $\times 1000$ .

FIG. 8. A multinucleated cell from the same preparation as the two preceding. As in Fig. 6, there is a darker area in the center of the cell suggesting the rosette area. This cell resembles a Langerhan's giant cell. Wright's stain.  $\times 1000$ .



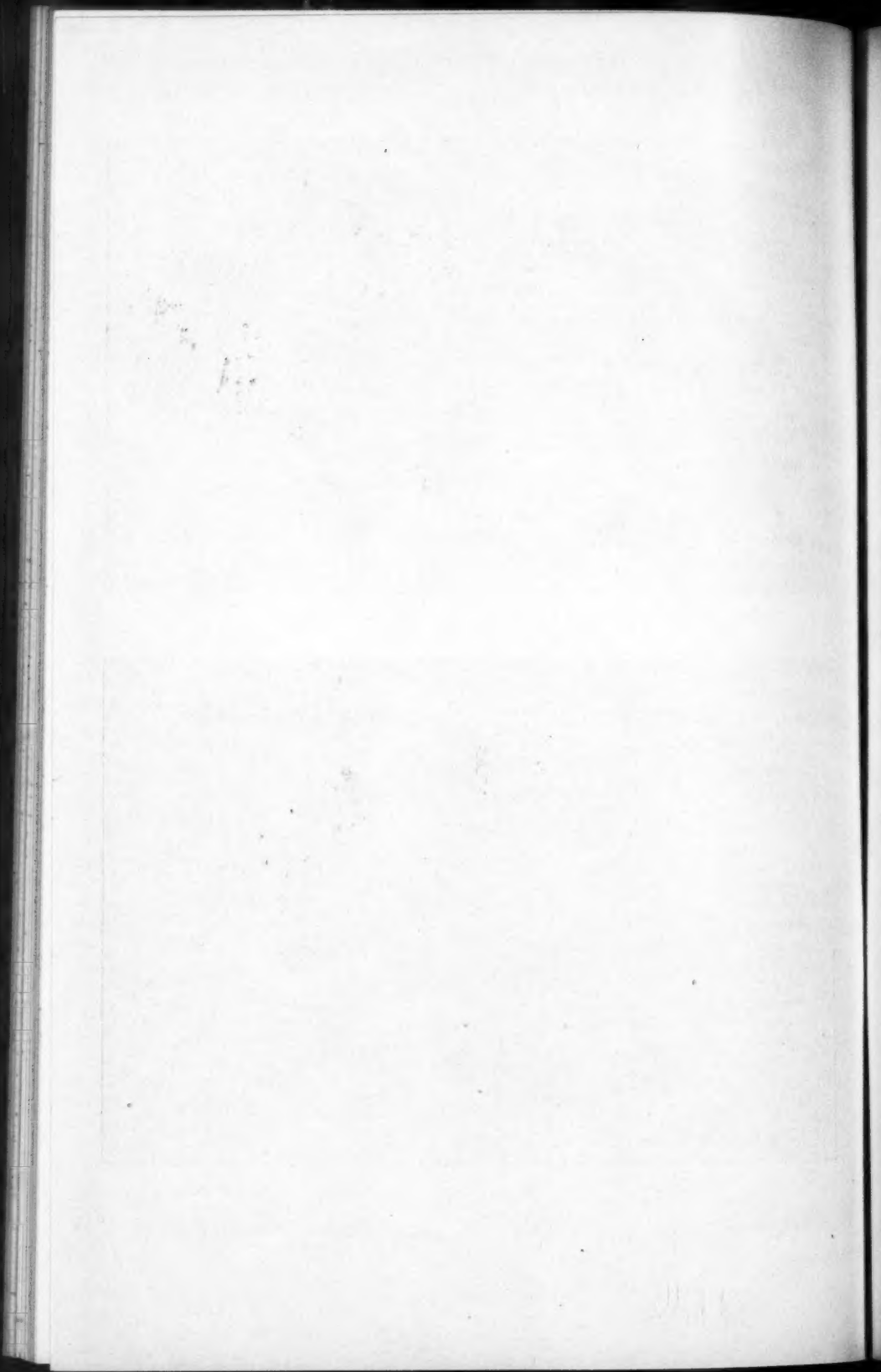
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Rhoads and Parker

Incubated Tissues and Exudates



## FIBROSARCOMA OF THE PLEURA\*

### REPORT OF A CASE

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A malignant tumor of fibroblastic origin arising in the pleura is very unusual. In reviewing the literature to 1908, Robbins<sup>1</sup> briefly epitomized ten cases of pleural sarcoma. These showed a wide variation both in their gross morphology and in their pathologic histology, and occurred in patients whose ages ranged from three to sixty-seven years.

Robbins reported a case in which a sarcoma formed a thin but distinct layer over the entire surface of the left pleural cavity. This tumor invaded the lungs and metastases were found in the mediastinal lymph nodes and in the liver.

In another case, reported in 1916 by Pallasse and Roubier,<sup>2</sup> a large fibrosarcoma filled the right pleural cavity and invaded the diaphragm. These authors offer a classification of primary malignant tumors of the pleura, dividing them into sarcomas and endotheliomas, diffuse and circumscribed, and point out that a distinction may not readily be drawn.

During the past ten years little attention has been given to this condition by either clinicians or pathologists.

### REPORT OF CASE

*Clinical History:* J. C. (Hospital number 544907), a white male, unmarried, fireman, 71 years of age, entered the Boston City Hospital on Aug. 13, 1927, complaining of a pain in the right side of his chest and shortness of breath. The family history is negative, and the past history, except for a loss of thirty pounds in weight during the preceding six months, is without interest.

His present illness apparently began about three weeks before entry when the patient was seized with an excruciating pain in the right side of his chest. This was relieved with rest and heat. After two or three days he noticed that on slight exertion he would become short of breath. Both of these symptoms persisted till the time of his admission into the hospital.

*Physical Examination:* An elderly man, well developed and well nourished. The findings, other than those relating to his chest, are irrelevant. The chest was asymmetrical; the movements, somewhat hurried and gasping, were rapid

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and shallow, with expiration moderately prolonged. The expansion on the right side was diminished. The left lung was resonant to percussion; the right, slightly resonant at the apex, was dull to flat over the remainder of the chest. Tactile and vocal fremitus on the right side were diminished over the greater portion of the lung and absent at the base. Breath sounds were exaggerated throughout the left lung, bronchovesicular over the right apex, and distant to absent from the level of the third rib down. There was a Grocco's triangle on the left side. The heart was enlarged or displaced. Apical impulse, distinctly palpable 15 cm. to the left of the midsternal line, was diffuse in character, but regular in force and rhythm. There were no murmurs. Blood pressure, systolic 100, and diastolic 65.

*Treatment and Progress Notes:* On day of admission, 1140 cc. of thin blood-stained fluid, with a specific gravity of 1.025, were aspirated from the right pleural cavity. The symptoms were appreciably relieved. Two days later, 300 cc. of dark, uniformly colored, bloody fluid, with specific gravity of 1.030, were removed from the same cavity. The patient's condition became worse and he died during the night.

*Laboratory Findings:* With the exception of the examination of the aspirated fluid the laboratory findings were negative. The first fluid showed 46 per cent lymphoid cells and 54 per cent polymorphonuclear leucocytes. Cultures and guinea pig inoculation were negative. Examination of the second specimen was negative for tumor cells.

*Clinical Diagnosis:* Cardiac decompensation; hydrothorax.

The autopsy was performed six hours postmortem. Tissue was immediately placed in Zenker's fixative and later embedded in paraffin and stained with eosin-methylene blue, phosphotungstic acid hematoxylin, and Mallory's aniline blue.

#### AUTOPSY REPORT

The right side of the chest is larger than the left. The right side of the scrotum is distended with a firm, solid, nodular, round mass, 8 cm. in diameter, which is neither adherent to the skin nor to the subcutaneous tissue.

*Peritoneal Cavity:* The diaphragm on the right is depressed to the level of the costal margin, whereas on the left it is at the fifth intercostal space. The lower margin of the liver is 11 cm. below the xiphoid process and 10 cm. below the costal margin in the right midclavicular line.

*Pleurae and Lungs:* The left lung and left pleura on gross examination are normal and may be passed without further comment. On the right, the parietal pleura is unevenly thickened and forms a large sac, measuring 30 by 20 by 20 cm., which is distended with 2200 cc. of thin blood-stained fluid. The lung is collapsed and forms

a small and very firm mass projecting from the medial wall of the pleural cavity.

The parietal and visceral pleurae, posteriorly and toward the mediastinum, are firmly adherent to one another. The visceral pleura, which is red and shaggy, is only slightly thickened except where small finger-like processes encroach upon the lung parenchyma. The inner surface of the parietal pleura is mottled yellowish brown and red, and presents a cobblestone appearance roughened by adhering flecks of fibrin. The fresh surface, which varies in thickness from 3 mm. to 4 cm., is firm, friable, glistening and gray. It strips easily from the ribs and intercostal spaces leaving a relatively smooth surface. Medially it is attached to the parietal pericardium, and inferiorly it is inseparably united to a thickened, hard and nodular diaphragm.

*Pericardial Cavity:* Surfaces are smooth and glistening. On the right side the pleura and pericardium are very adherent.

*Heart:* Weight 435 gm., is moderately enlarged and displaced to the left. The wall of the left ventricle is thickened; the coronary arteries are sclerosed.

*Mediastinum:* The mediastinal lymph nodes are normal in size, soft and deeply pigmented. A single node containing a minute, hard, white, glistening body, 2 mm. in diameter, lies adjacent to the thickened right pleura.

The remaining autopsy findings include moderate and generalized arteriosclerosis, a congenital cystic kidney, and an old infectious lesion of the right testicle.

The microscopic examination of the right pleura, right lung, peribronchial lymph nodes and diaphragm is of particular interest.

#### MICROSCOPIC EXAMINATION

*Pleura:* The entire right parietal pleura is infiltrated by tumor tissue composed of round and spindle-shaped cells and a variable amount of intercellular material. The morphology of the cells, their arrangement, their invasive character and their rapidity of growth suggest a fibrosarcoma. Toward the ribs and intercostal spaces, the tumor is very cellular and is invading the surrounding fat and fibrous tissue; here the cells are growing rapidly and forming very little intercellular substance. Nearer the pleural cavity where the tumor is growing more slowly the ratio of cells to collagen is re-

versed, there being few cells and much collagen. The inner surface of the cavity is composed of necrotic tissue covered by a layer of fibrin and poorly preserved inflammatory cells.

The structure of the visceral pleura varies in different places and generally is quite unlike the parietal layer. Over the greater part of the surface of the lung it is represented as a relatively thin and uneven layer of poorly preserved collagen with a few degenerating fibroblasts and endothelial leucocytes. In these areas the collagen has formed coarse strands which cross one another, run in different directions, and show no orderly arrangement.

In some areas where a continuous layer of elastic fibrils still indicates the position of the original pleura, collagen showing this same disorderly arrangement has been deposited on both sides of the pleura.

In a few places along the surface there is actively growing tumor tissue which has invaded the lung parenchyma. In such areas it is usually found that as the tumor is traced into the lung it gradually loses its cellularity and becomes more and more collagenous.

Lastly there are portions of the lung that are covered with a well preserved and orderly layer of hyaline connective tissue enclosing few foci of lymphoid cells and endothelial leucocytes.

*Lung:* The entire lung shows a varying degree of atelectasis, which is greatest at the periphery beneath the thickened pleura. The parenchyma of the posterior and inferior portion of the lower lobe is deeply invaded by actively growing tumor tissue. In this region the bronchioles are compressed and the alveoli are completely collapsed or have totally disappeared. There is a cellular infiltration composed largely of lymphoid cells and endothelial leucocytes throughout this portion of the lung, but this inflammatory reaction is most concentrated along the advancing margin of the tumor.

The alveolar spaces of the middle and upper lobes contain little precipitated albumen, few red blood cells, pigmented endothelial leucocytes and threads of fibrin. As a result of a hemorrhage, the alveoli of the lower lobe are filled with well preserved red blood cells.

Small patches of pneumonia in which the bronchioles and surrounding alveoli are filled with an acute inflammatory exudate are found in all three lobes.

The vessels everywhere are very congested and the walls of the larger arteries are thickened and sclerosed. The perivascular and

peribronchial lymphatics are distended with polymorphonuclear leucocytes, lymphoid cells and endothelial leucocytes. Many foci of lymphocytes lie in the scar tissue surrounding the larger bronchioles.

A rather unusual finding in the lung, and one which probably bears no relation to the sarcoma of the pleura, is the presence of a small group of alveoli, situated at some distance from the tumor, which are distended with blocks of cartilage of a primitive type suggesting a small chondroma.

*Lymph Node:* A small focus of tumor tissue is situated in the periphery of a single node and is extending by finger-like processes into the central portion. Here the tumor is growing rather slowly, there are very few mitoses and the cells are well differentiated and separated by a considerable amount of intercellular material. The lymph nodules show a rather aplastic condition while the sinuses are filled with endothelial leucocytes containing carbon pigment and hemosiderin.

*Diaphragm:* This is covered on the upper surface by a narrow layer of actively growing tumor tissue and in a few places is definitely invaded. The muscle fibers beneath and surrounded by the tumor are compressed; they are small, atrophic and degenerated and many have completely disappeared with consequent sclerosis of the surrounding stroma. Throughout the diaphragm there is a well marked chronic inflammatory reaction which is most marked near the tumor.

#### HISTOLOGY OF THE TUMOR

The type cell from which the tumor arises is the fibroblast. In this tumor it occurs most commonly as a long, well differentiated spindle-shaped cell. The nucleus is elongated, the chromatin forms a very delicate network, and the nucleoli appear as small round dots. Less frequently the cell is nearly round or oval; this type is probably less differentiated and is formed where the tumor is growing most rapidly; here, the nucleus is round or irregular, the network of chromatin is coarse, and the nucleoli vary in number, in size, and in contour. Between these two rather characteristic types there are many polymorphous forms. Single and multiple mitoses are fairly numerous.

Tumor giant cells, containing a single large nucleus or many nuclei, are sparsely distributed in the more cellular portions of the

tumor. Their nuclei show a wide variation in size and in shape. The chromatin is coarsely granular, and the nucleoli vary from small discrete dots to large irregular bodies. The cytoplasm of these cells stains more deeply than the smaller cells and extends out in streamers in many directions.

Fibroglia fibrils may be traced from the ends of the spindle-shaped cells, but they are not visible where the cells are more primitive and polymorphous.

Collagen fibrils, which form the intercellular matrix, vary in different parts. Where the tumor is growing actively and invading there is very little intercellular material, whereas in other areas where growth has practically ceased there is a great deal of collagen, and the fibrils have fused together to form wide irregular bands.

The grouping of the cells is inconstant. Sometimes the spindle-shaped cells lie in parallel rows, more commonly they are grouped in bands which course in any direction. The less mature cells show no definite arrangement except when clustered about the vessels.

The stroma in the cellular portion of the tumor consists of dilated blood vessels lined with a single layer of endothelium. In the more collagenous portions the vessels appear compressed and are surrounded by a definite ring of connective tissue.

Small areas are dispersed throughout the tumor in which the cells are undergoing degeneration or are already necrotic. These areas are always infiltrated with polymorphonuclear and endothelial leucocytes, and frequently, in the zones of necrosis, there is some hemorrhage and blood pigment suggesting a gradual infarction.

#### DISCUSSION

Clinically it seems remarkable that a tumor could extend throughout the entire pleura, produce such an effusion, and lead to such a degree of atelectasis without producing more discomfort, since from the clinical history it is apparent that we are describing a patient who had no symptoms until three weeks before entry into the hospital. Furthermore, the clinical impression of the case as being one of cardiac decompensation and hydrothorax suggests the difficulty of diagnosis.

From the view point of the pathologist the tumor is unusually interesting, first because a fibrosarcoma of the pleura is very rare,

and secondly, because one forming a complete sac of the pleural cavity is indeed a curiosity.

It would be difficult to suggest how or from what part of the pleura this tumor had originated. There is some evidence that there has been an old healed pleuritis since many portions of the thickened visceral pleura may be readily considered as simply the result of an old inflammatory process.

The most actively growing portion of the tumor is along the outer surface of the parietal pleura, whereas along the internal surface of the parietal pleura and in the visceral pleura growth has almost ceased. It was pointed out above, that small areas are scattered throughout the more cellular portion of the tumor which show degenerative changes frequently associated with some hemorrhage and blood pigment. Probably these are simply early retrograde changes in the cells secondary to a diminished circulation, and such an explanation is consistent with the varied histologic pictures which different portions of the tumor reveal.

In general, the tumor may be classed as a slowly growing fibrosarcoma showing little tendency either to invade or to produce metastases.

#### SUMMARY

1. A case is reported of a primary fibrosarcoma of the pleura in an elderly male.
2. The literature is briefly reviewed.
3. The case is discussed as an unusual pathologic condition which warrants clinical consideration.

We are indebted to Dr. F. B. Mallory and Miss Catherine G. Norton for the illustrations and to Miss Marion E. Lamb for technical assistance.

#### REFERENCES

1. Robbins, W. B. Primary sarcoma of the pleura. *Boston M. & S. J.*, 1908, clviii, 691.
2. Pallasse, E., and Roubier, C. Les tumeurs primitives de la plèvre. *Ann. de. med.*, 1915, iii, 243.

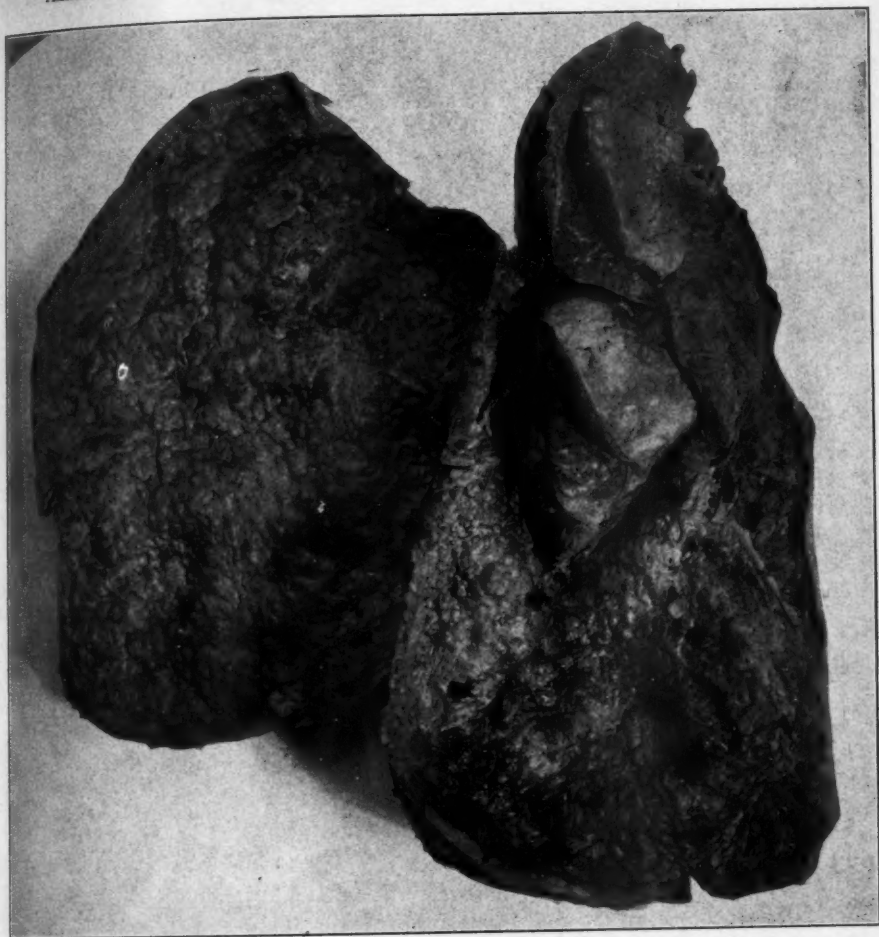
## DESCRIPTION OF PLATES

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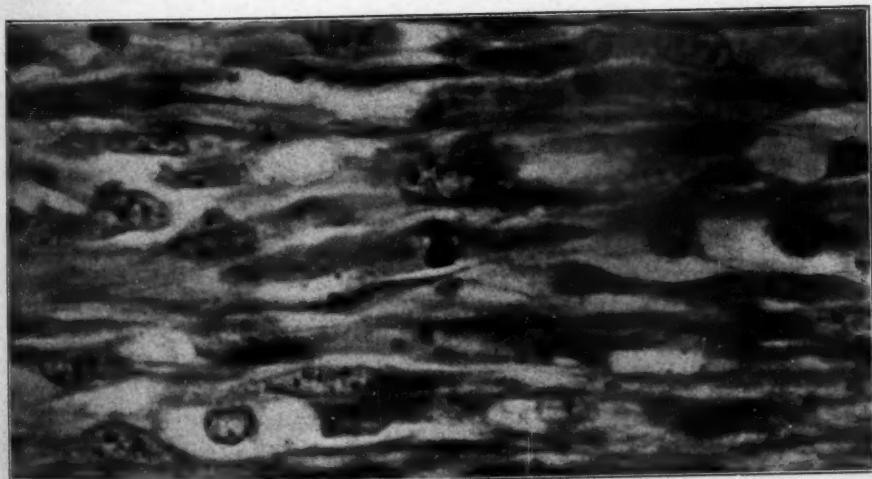
### PLATE 91

FIG. 1. The right pleura has been incised and the halves spread out to show the cobblestone appearance of the inside of the cavity, and the small atelectatic lung.

FIG. 2. Portion of the tumor showing the spindle-shape morphology of the cells, and their parallel arrangement. One mitotic figure.  $\times 1000$ .



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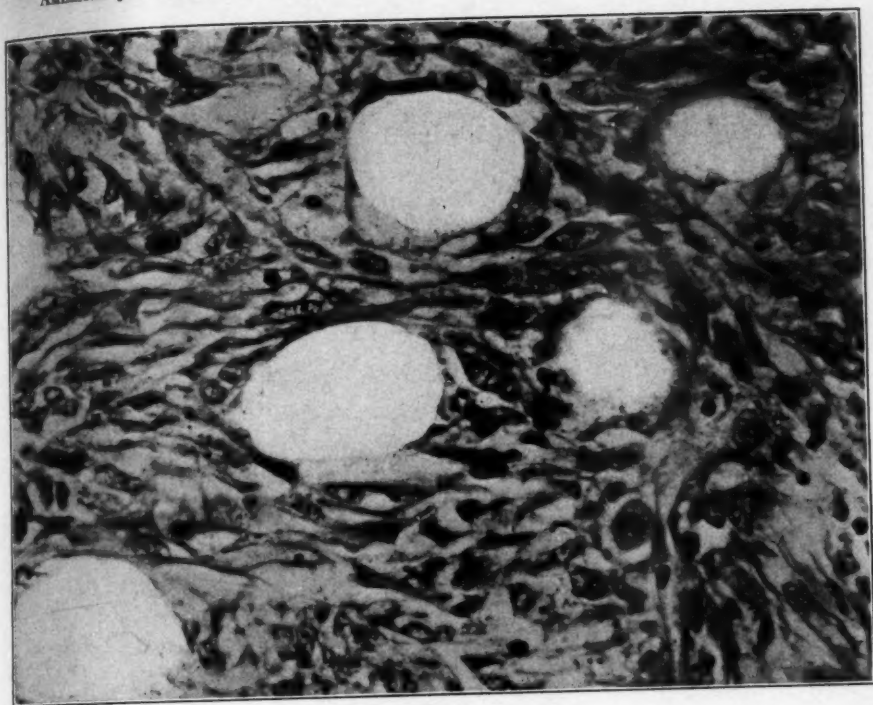
MacMahon and Mallory

Fibrosarcoma of Pleura

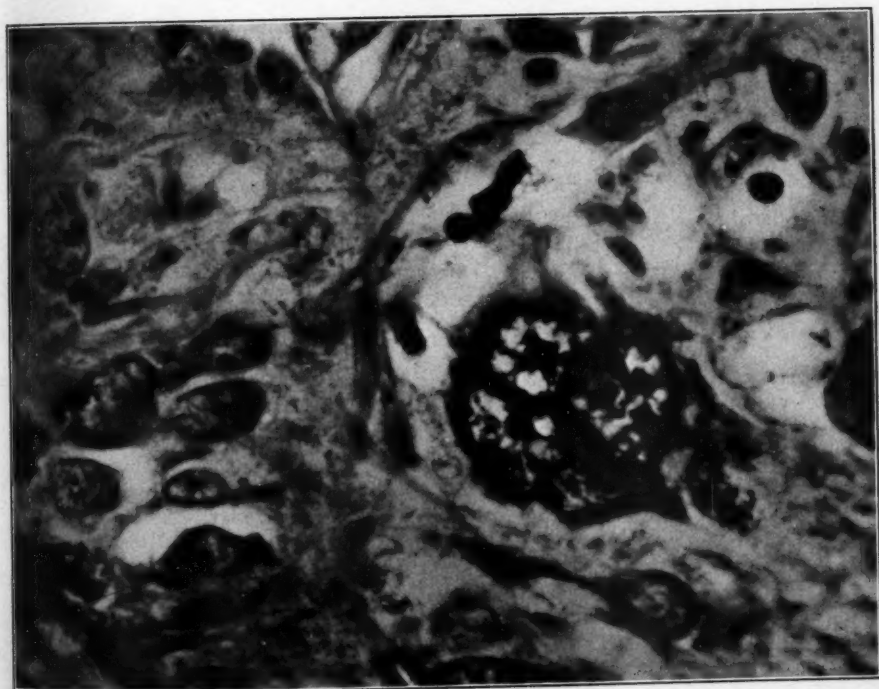
PLATE 92

FIG. 3. Invasion of fat by tumor. An actively growing portion of the tumor showing several mitoses.  $\times 500$ .

FIG. 4. An area in which the cells are oval and round. Single and multinucleated giant cells.  $\times 1000$ .



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